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HEPATITIS C VIRUS PREVALENCE IN PEDIATRIC NON-HODGKIN LYMPHOMA

Thesis
Submitted in partial fulfillment for the requirements of the Master Degree in Pediatrics

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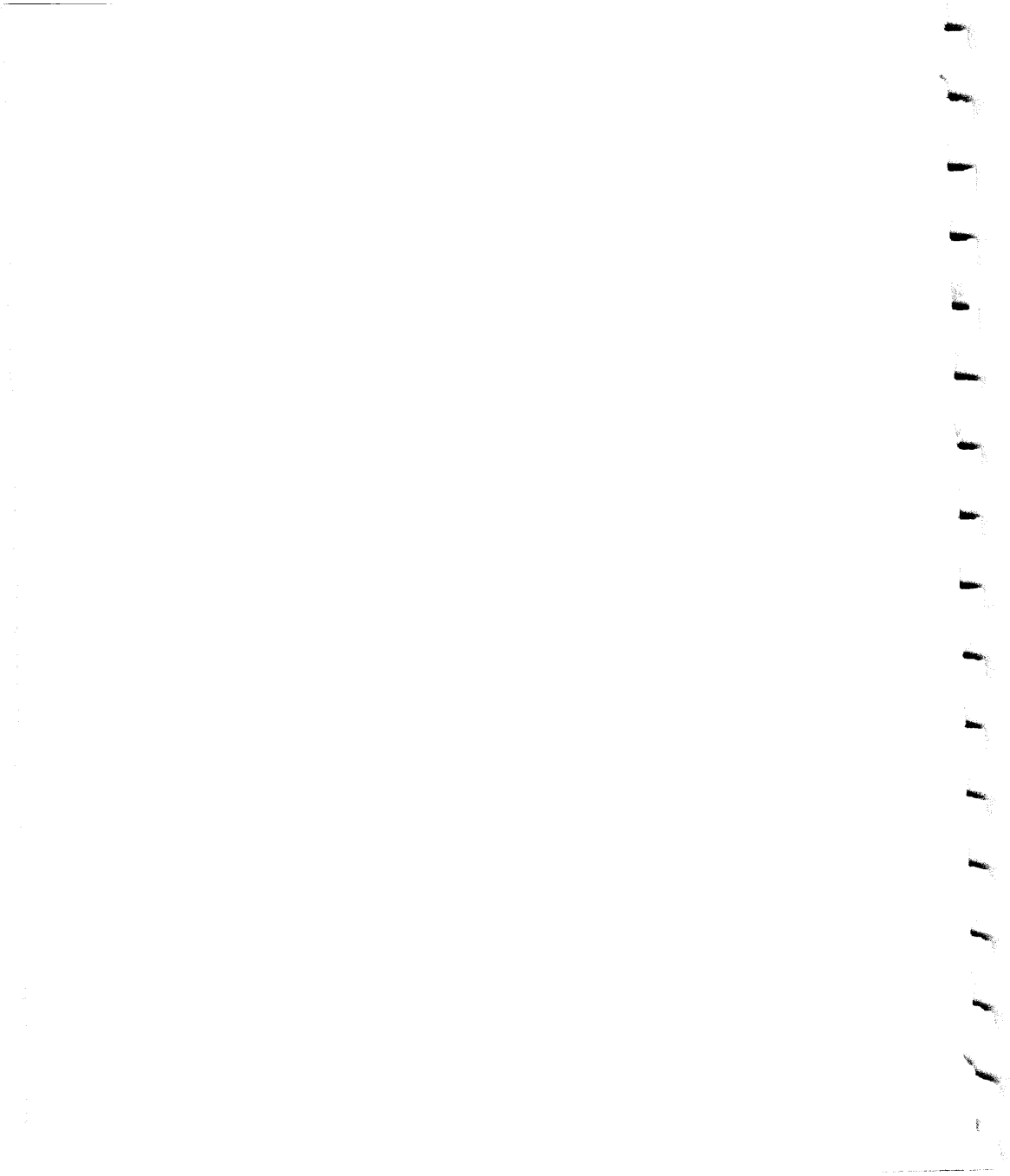

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Mohamed El Naghi
2006

Dedication

This work is dedicated to the individuals who have given meaning to my life;

To my **parents,** who gave me every thing and took nothing.

To my **sisters**, who helped me in every step of my life.

To my colleagues in MUCH who helped me to complete this work.

Mohamed El Naghi
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Abbreviations

Ag	Antigen
AIDS	Aquired Immunodeficiency Syndrome
ALT	Alanine transaminase
AML	Acute myeloid leukemia
Anti-HBc	Antibody of Hepatitis B core
Anti-LKM-1	Antibodies to actin and to liver/kidney microsomes
AST	Aspartate transaminase
BFGF	Basic fibroblast growth factor
BMT	Bone marrow transplantation
BUN	Blood Urea Nitrogen
CD	Cluster of differentiation
CDC	The Centers for Disease Control
CNS	Central nervous system
CT	Computed tomography
CXR	Chest X-ray
CYP	Cytochrome P450
DLBCL	Diffuse large B-cell NHL
DNA	Deoxyribo nucleic acid
EBV	Ebstien Barr Virus
ECOG	Eastern Cooperation Oncology Group
EIA	Enzyme Immuno Assay
EMC	Essential Mixed Cryoglobulinemia
FDA	Food and Drug Adminstration
FDG	Fluorodeoxyglucose
Fig	Figure
FISH	Fluorescent in situ hybridization
FNA	Fine Needle Aspiration
GEP	Gene Expression Profiling
GI	Gastrointestinal
GST	Glutathione S-Transferase.
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C virus
HD	Hodgkin's disease
HIV	Human Immundeficiency Virus

HTLV	Human T-cell Lymphotropic Virus
HVR	Hypervariable region
IFN	Interferon
IGF	Insulin like growth factors
IHC	Immunohistochemistry
IL	Interleukin
IMPDH	Inosine Monophosphate Dehydrogenase
IPI	International Prognostic Index
IRES	Internal ribosomal entery site
ITP	Idiopathic thrombocytopenic purpura
IU	International unit
LDH	Lactate dehydrogenase
LP	Lichen Planus
mAbs	Monoclonal antibodies
MALT	Mucosa-associated lymphoid tissue
MG	Myasthenia gravis
MP-NAT	Minipool nucleic acid testing
MRI	Magnetic resonance imaging
MUCH	Mansoura University Children Hospital
NAT	Nucleic acid testing
Nc	Noncoding
NHL	Non Hodgkin Lymphoma
NK	Natural killer
NO.	Number
NSl-NS6	Non structural protiens 1- 6
ORF	Open reading frame
P	Propability value
PAT	Parenteral antischistosomal therapy
PBSC	Peripheral blood stem cell (PBSC)
PCT	Porphyria cutanea tarda
PET	Positron emission tomography
REAL	Revised European-American Lymphoma
RIBA	Recombinant ImmunBlot Assay
RNA	Ribonuceic acid
RQ-PCR	Real time Quantitative polymerase chain reaction
RT	Reverse transcription

SGOT	Serum glutamic oxalacetic transaminase
SGPT	Serum glutamic pyrovate transaminase
SiRNAs	Small interfering RNAs
SPECT	Single photon emission computed tomography
SVR	Sustained Viral Response
TNF	Tumour necrosis factor
TNM	Tumor – Lymph Nodes- Metastasis
U/S	Ultrasonography
USA	United States of America
VEGF	Vascular endothelial growth factor
WF	Working Formulation
WHO	World Health Organization

Contents

List of Tables

List of Figures

Introduction And Aim Of The Work

Introduction

The World Health Organization (WHO) estimated that 170 million people are infected with hepatitis C virus (HCV) ***(Lavanchy et al., 2000).*** An estimated 12- 15% of Egyptians, or 8–10 million people, have serological evidence of HCV infection, with higher rates in older age groups and residents of rural areas in Lower and Middle Egypt. There is compelling evidence for a large-scale iatrogenic transmission of HCV during a parenteral anti-schistosomal treatment campaign carried out from the 1920s through the 1980s ***(Frank et al.,2000).*** Continued transmission in Egypt has been associated with transfusion of unscreened blood; invasive medical procedures, including Caesarean section and abortion; injections by informal health care providers***(Habib et al. ,2001)***; and haemodialysis ***(Alter et al., 2000).***

Hepatitis C virus (HCV) is a small single stranded RNA virus identified in 1989 ***(Choo et al., 1989),*** which belongs to the family of flaviviridae ***(Lauer et al., 2001).*** The HCV expresses its genetic information in the form of a single large polyprotein of about 3000 aminoacids encoded by an open reading frame (ORF) that extends most of its genomic RNA. Proteolytic cleavage of the ORF product into multiple structural and nonstructural peptides is essential for the virogenesis .

Involvement of lymphoid cells in HCV infection (HCV lymphotropism) can provide insight into the pathobiology of extrahepatic dissemination of HCV ***(Zignego et al., 1992).*** Extrahepatic sites of HCV may also act as a source of continuous infection with HCV and reinfection of hepatocytes. Regarding haematological diseases related to HCV infection a large number of data encourages the *"Haematology-Hepatology linkage".*

One of the extrahepatic diseases in which HCV has been implicated is B-cell non-Hodgkin's lymphoma (NHL). HCV associated lymphomas have been observed, but whether they are caused by HCV remains to be shown definitively. There is a suggestion that some B-cell NHL associated with HCV arise from clonal expansion of B-cells with particular immunoglobulin gene rearrangements specific for the E2 protein of the HCV envelope ***(Ivanovski et al., 1998;Quinn et al., 2001)***, which is consistent with the hypothesis that lymphomas develop when B cells proliferate in response to antigen. However, no biological mechanism of HCV-associated lymphomagenesis has been definitively elucidated.

The relationship between HCV infection and lymphoproliferative diseases was first reported by ***Ferri et al in 1994*** and was subsequently highlighted by other Italian ***(Vallisa et al., 1999)***, Japanese ***(Izumi et al., 1997)***, American ***(Zuckerman et al., 1997)*** and Turkish ***(Timuraglu et al., 1999)*** authors, with the prevalence of HCV infection ranging from 9% to 42%. Conversely, research carried out in Great-Britain ***(McColl et al., 1996)***, Canada ***(Collier et al., 1999;Shariff et al., 1999)*** and the USA ***(King et al., 1998)*** did not identify a relationship between HCV and lymphoma, suggesting that the prevalence, environmental and ethnic factors, the distribution of the different virus genotypes, mutations and other infectious agents could account for the geographic variation ***(Hanley et al., 1996;McColl et al., 1997)***.

Aim of the work

In this study we aimed to:

1- Determine the prevalence of hepatitis C virus infection in children with Non-Hodgkin's lymphoma.

2- Investigate the relation between HCV and lymphoproliferative diseases in children.

3- Provide epidemiological data for Egypt regarding this issue.

Working in a population with high prevalence of HCV would allow to conduct a case-control study with adequate statistical power to assess the question of whether there is an association of chronic HCV infection with NHL in egyptian children.

Hepatitis C Virus

I-History and nature

It became apparent after the discovery of the hepatitis A and B viruses in the late 1960s and early 1970s that a large proportion of cases of acute and chronic hepatitis could not be explained by either of these agents. Another viral agent was suspected, and patients infected with this suspected agent were said to have non-A, non-B hepatitis. The agent was finally identified by genetic cloning and not by isolation in 1989 when the genome of the virus was cloned and the agent was designated the hepatitis C virus (HCV) ***(Choo et al., 1989).*** Six years after the initial publication knowledge on HCV has dramatically improved with regard to the genomic structure and variability of the virus in contrast, morphologic and viral growth data are still in their early years ***(VanDoorn et al., 1994)***.

HCV is closely related to flaviviruses and pestiviruses. Its genetic organization and protein products classify it in the flaviviridae family, although its diversity is great enough for it to be classified as a separate genus . HCV is not related to any of the other known hepatitis viruses ; however , the recently described hepatitis G virus is a distant relative ***(Sakai, 1999).***

II-Viral Heterogeneity

The polymerase enzyme of RNA viruses such as HCV lacks proofreading ability and is therefore unable to correct copying errors made during viral replication. Many of these nucleotide changes result in a nonfunctional genome or a replication incompetent virus (lethal mutants). However, others persist and account for the tremendous viral diversity that is characteristic of HCV. This heterogeneity is extremely important in the diagnosis of infection, pathogenesis of disease, and the response to treatment; it prevents the development of conventional vaccines, allows the virus to escape eradication by the host's immune system, and affects the completeness of the response to antiviral therapies such as interferon (***Farci et al., 1992***). Viral heterogeneity takes several forms depending upon the degree of diversity:

Quasispecies are families of different, but highly similar, strains that develop within an infected host over time. Nucleotide sequence homology is greater than 95 percent.

Over decades and centuries, the degree of HCV diversity has evolved into several distinct genotypes of the virus ***(Simmonds, 1995)***. Sequence homology between genotypes is less than 80 percent. There are six genotypes and numerous subtypes of HCV.

III-Quasispecies

Differences between quasispecies families are usually only apparent in the most rapidly changing parts of the genome (hypervariable regions). Typically, a single dominant sequence changes with time, being replaced by one or more minor populations due to external pressures (e.g., the host immune system) on the quasispecies. One group of investigators generated more than one hundred clones of the most genetically heterogeneous region of a single HCV isolate; they found 19 unique sequences, confined largely to the first hypervariable region (HVR1) of the E2 envelope protein ***(Farci et al., 1996).***

Patients who had a sustained response to interferon had less pretreatment quasispecies heterogeneity than those who either relapsed or had no response to treatment. Another report, in which HCV quasispecies were determined in liver samples, found that different quasispecies were compartmentalized within specific regions in the liver, and that the degree of compartmentalization was greater in histologically advanced disease ***(Sakai et al., 1999).***

This genetic diversity of HCV may allow it to escape host immune surveillance, thereby resulting in virus persistence and a lack of protective immunity ***(Farci et al., 1992; Weiner et al., 1992; Farci et al., 1996).*** In one study, for example, markers of viral replication and host immunity were studied in five chimpanzees sequentially inoculated over a period of three years with different HCV strains ***(Farci et al., 1992).***

Each rechallenge of a convalescent chimpanzee with the same or a different HCV strain resulted in reinfection. Reinfection has also been observed in multiple transfused patients ***(Lai et al., 1994).*** The genetic diversity of HCV has obvious implications for vaccine development.

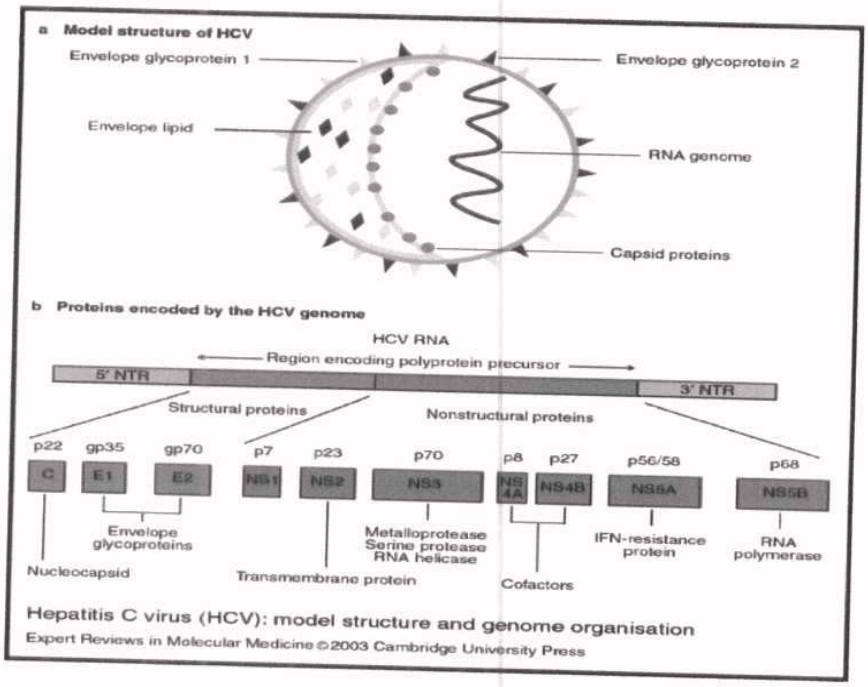
IV-VIRAL GENOME AND REPLICATION:

On the basis of their genome organization and vision properties; Properties of Flaviviridea family include a lipid envelope and a single stranded, positive polarity RNA genome which contains a long open reading frame (ORF) which encodes the viral polyproteins, the structural proteins are located at the N-terminal portion followed by the putative non-structural components ***(Grakoui et al., 1993).***

For flaviviruses, the development of neutralizing antibodies is correlated with protective immunity, while in the case of HCV reinfection, cross-challenge studies have demonstrated very little protective immunity ***(Farci et al., 1992)***.

HCV differs from its relatives and indeed, from most other RNA viruses, in the high efficiency with which it establishes chronic infection ***(Ralston et al., 1993).*** The HCV genome is a positive-sense RNA molecule of approximately 9500 nucleotides. There are highly conserved 5' and 3' untranslated regions flanking an approximately 9000 nucleotide single open reading frame which encodes a large polyprotein of about 3000 amino acids ***(Major et al., 1997)***. This protein undergoes posttranslational processing by host and viral enzymes to form the structural and nonstructural proteins and enzymes of the virus.

Fig.(1):Model structure and genome organisation of HCV.



(Mónica et al., 2003) .

Fig.(1)

(a) *Model structure of HCV. The left-hand side of the illustration shows the viral surface of envelope lipids and glycoproteins; the right-hand side shows the RNA genome encased by capsid proteins.*

(b) Proteins encoded by the HCV genome. HCV is formed by an enveloped particle harbouring a plus-strand RNA of ~9.6 kb. The genome carries a long open-reading frame (ORF) encoding a polyprotein precursor of 3010 amino acids. Translation of the HCV ORF is directed via a ~340 nucleotide long 5' nontranslated region (NTR) functioning as an internal ribosome entry site; it permits the direct binding of ribosomes in close proximity to the start codon of the ORF. The HCV polyprotein is cleaved co- and post-translationally by cellular and viral proteases into ten different products, with the structural proteins [core (C), E1 and E2] located in the N-terminal third and the nonstructural (NS2–5) replicative proteins in the remainder. Putative functions of the cleavage products are shown ***(Mónica et al., 2003).***

V-Genotypes

Six major genotypes of HCV have been defined ***(Simmonds et al., 1995)***. More than 50 subtypes have also been described; the most common subtypes are 1a, 1b, 2a, and 2b. the evolution of genotypes has probably been influenced by several factors, including immune selection, infection patterns, replication efficiency, and population migration. Thus, there is a distinct geographic distribution of HCV genotypes ***(Dusheiko et al., 1994; Lau et al., 1996).***

Genotype 1 is most common (60 to 70 percent of isolates) in the United States and Europe ***(Lau et al., 1996, Dusheiko et al., 1994)***; genotypes 2 and 3 are less common in these areas, while genotypes 4, 5, and 6 are rare:

- Genotype 3 is most common in India, the Far East, and Australia.
- Genotype 4 is most common in Africa and the Middle East .
- Genotype 5 is most common in South Africa
- Genotype 6 is most common in Hong Kong.

The clinical significance of viral genotypes is not entirely clear, but it appears to have a significant effect upon the response to interferon-based therapy. The sustained virologic response to pegylated interferon plus ribavirin ranges from about 40 to 50 percent with genotype 1 (including 1a and 1b) to as high as 70 to 80 percent with genotypes 2 and 3 ***(Martinot et al., 1995; Davis et al., 1997).***

VI-Epidemiology

PREVALENCE:

Egypt is one of the highest HCV prevalence in the world; 10%-20% of the general population are infected and HCV is the leading cause of HCC and chronic liver disease in the country ***(Arthur et al., 1997; Nafeh et al., 2000; El-Zayadi et al., 2001; Habib et al., 2001)***. Approximately 90% of Egyptian HCV isolates belong to a single subtype, 4a, which responds less successfully to interferon therapy than other subtypes ***(El- Zayadi et al., 1996; Angelico et al., 1997; Kamal et al., 2000; Ray et al., 2000)***. Furthermore, HCV is less prevalent in countries neighboring Egypt that have similar sociomedical conditions and similar HCV strains ***(McCarthy et al., 1994; Shobokshi et al., 1999).***

Why, then, is Egypt so seriously affected? Previous research has suggested that the Egyptian HCV epidemic results from the use of unsterile injection equipment during mass treatment of the general population with parenteral antischistosomal therapy (PAT) ***(Quinti et al., 1995; Frank et al., 2000)***. Cross-sectional epidemiological analyses have provided evidence for the PAT hypothesis; there is a correlation between the level of exposure to PAT and HCV prevalence among different age groups and geographic regions ***(Angelico et al., 1997; Frank et al., 2000; Nafeh et al., 2000; Habib et al., 2001).***

Although subtype 4a is the dominant Egyptian HCV strain, a survey by of HCV genetic diversity in the country revealed that other subtypes (provisionally named 4a, 4b, and 1g) are also present at lower prevalences. The most recent common ancestor of each subtype (including 4a) existed approximately 80–120 years ago, suggesting the recent and simultaneous appearance of a few pre-existing endemic strains. This is in contrast to the

pattern of HCV genetic diversity found in other developing regions, which is more consistent with a long period of endemic infection ***(Ray et al., 2000).***

The outcome of HCV infection acquired in childhood is even more uncertain because of the diversity of the epidemiological and clinical features of infection and disease in children ***(Tovo et al., 2000).***In Egypt, the population-based HCV prevalence is about 18%. Community-based data suggest that some 17.5% of HCV-infected Egyptians are children.

Two studies from upper and lower Egypt revealed that the prevalence of HCV antibody (anti-HCV) in children is 3 and 9%, respectively ***(Habib et al., 2001).***

Little data are available about disease morbidity. Although the prevalence of HCV infection is lower in children than in adults, the pediatric hepatologist only deals with the beginning of a chronic illness that might take a more sinister progressive course in the long run ***(Hardikar, 2002).***

Modes of Transmission in Children:

1. **Blood transfusion**: Blood transfusion was a major risk -for acute infection in the past, with more than 10 percent of transfusion recipients acquiring infection in some studies ***(Alter et al., 1989).*** The screening of blood donors for historical risk factors, serologic evidence of hepatitis B infection (HBsAg and anti-HBc), and elevated serum ALT caused a striking reduction of non-A, non-B post-transfusion hepatitis, even before HCV was identified.

The subsequent initiation of donor screening for anti-HCV antibodies in 1990 has nearly eliminated the risk of post transfusion acute HCV infection. The estimated risk is now 1:100,000, with the remaining small risk probably being due to recent acquisition of HCV infection by the donor prior to appearance of anti-HCV antibodies ***(Schreiber et al., 1996).***

In the spring of 1999, another blood donor HCV testing technology, nucleic acid testing (NAT), was introduced ***(Tabor et al. 2002).*** It is anticipated that this technology, which detects HCV genetic material rather than later-appearing antibodies, will further decrease the risk of transfusion-transmitted HCV to a range between 1:500,000 and 1:1,000,000 units, a 5- to 10-fold reduction ***(Schuttler et al., 2000)***. This continuing decline in risk of post transfusion HCV explains the observation that a history of blood product transfusion is much more common in patients presenting with chronic HCV (remote infection) than with acute infection.

Multiply transfused patients, including those with thalassemia or hemophilia, have been at particularly high risk of developing hepatitis C. The prevalence of anti-HCV in hemophiliacs who regularly received concentrates of clotting factors before adequate procedures were used to inactivate viruses (e.g., heat inactivation or pasteurization) was 84 to 100 percent ***(Mauser et al., 1995)***. Since the use of treated or recombinant clotting factors has become routine, new cases of hepatitis C infection have become uncommon in these patients. The continued high prevalence of anti-HCV in this population is due to past exposure to untreated concentrates ***(Troisi et al., 1993; Mauser et al., 1995).***

2. **Injection drug use**: Parenteral exposure to the hepatitis C virus is the most efficient means of transmission. Thus, it is not surprising that injection drug use with shared needles or other paraphernalia has

been the most common identifiable source of acute HCV infection in the United States ***(Garfein et al., 1996).***

The efficiency of transmission via this route is evidenced by the high rate of anti-HCV antibodies observed in short-term injectors. In one report, analysis of blood from 716 injection drug users found that the seroprevalence of HCV was 64.7 % among those who had injected for one year or less ***(Garfein et al., 1996).*** By comparison, the seroprevalence of hepatitis B virus, HIV, and HTLV was 49.8, 13.9, and 0.5 percent, respectively. HCV infection also has been associated with a history of intranasal cocaine use, presumably due to blood on shared straws.

3. **Perinatal transmission**: Perinatal transmission of HCV occurs at the time of birth in about 2- 5 % of infants born to anti-HCV positive women. HIV coinfection, which more than quadruples the risk of vertical transmission (approximately 19 versus 4 percent) ***(Yeung et al., 2001).***

- A high HCV viral load; mothers with viral loads higher than 1 x 10(5) are more likely to transmit hepatitis to their infants ***(Terrault et al., 1998).***
- The effect of HCV genotype on vertical transmission is uncertain ***(Mazza et al., 1998).***
- Breast feeding appears to be safe except in women who are coinfected with HIV ***(Lin et al., 1995; Polywka et al., 1999; ACOG committee opinion. 1999).***
- Studies of the effect of mode of delivery on perinatal transmission currently are inconclusive ***(Lin et al., 1994).***

Treatment of pregnant women or unusual precautions to reduce the risk of transmission is not recommended at this time. Early diagnosis of infection in newborns requires HCV-RNA testing since anti-HCV

antibodies are passively transferred from the mother. The American Academy of Pediatrics recommends screening all infants born to HCV-infected mothers ***(American Academy of Pediatrics, 1998).***

4. **Hemodialysis**: The incidence and prevalence of HCV infection among patients on dialysis has steadily declined in recent years. Among member nations in the European Dialysis and Transplant Association, for example, the prevalence of anti-HCV declined from 21 percent in 1992 to 17.7 percent in 1993 ***(Geerlings et al., 1994).*** Nonetheless, the 0.4 to 15 percent incidence of anti-HCV positivity in hemodialysis units continues to be a cause for concern.

Unresolved debate continues as to whether transmission of HCV in hemodialysis units may be affected by routine testing for anti-HCV antibodies, patient isolation, use of dedicated machines, and a ban on dialyzer reuse. The Centers for Disease Control and Prevention in the United States (CDC) does not recommend dedicated machines, patient isolation, or a ban on reuse in hemodialysis patients with HCV infection ***(Alter et al., 1989).*** However, strict adherence to "universal precautions," careful attention to hygiene, and strict sterilization of dialysis machines is recommended. Conventional cleansing and sterilization appear to be adequate to inactivate the virus. Unfortunately, eliminating the spread of HCV infection in hemodialysis units may require the development of treatments to eradicate the virus or vaccines to prevent infection.

5. **Organ transplantation**: Transplant recipients who receive organs from HCV-positive donors have a high risk of acquiring HCV infection and liver disease ***(Pereira et al., 1991;Roth et al., 1994; Pfau et al., 2000)***. Some studies have shown nearly universal transmission; in one report, 75 percent of the 29 recipients of organs (19 kidneys, 6 hearts, and 4 livers) from 13 anti-HCV positive donors

became anti-HCV or HCV-RNA positive ***(Pereira et al., 1991).*** Others have not found quite as strong an association; only 13 of 46 (29 percent) recipients of RIBA-positive donors developed post transplant liver disease in a second series, although HCV-RNA was not checked ***(Roth et al., 1994).***

Despite the latter study, concern over the risk of HCV transmission is great. Most procurement organizations and transplant centers have now developed policies for screening and selective utilization of organs from anti-HCV positive donors ***(Kiberd et al., 1994).***

6. **Other**: Other rare sources of percutaneous transmission of HCV include contaminated equipment used during the performance of procedures. As an example, HCV has been transmitted during colonoscopy ***(Bronowicki et al., 1997).*** Procedures involved in traditional medicine, folk medicine (e.g., scarification, cupping),tattooing ***(Haley et al., 2001),*** body piercing, and commercial barbering may also transmit HCV on rare occasions.

VII-Clinical Disease

The incubation period of hepatitis C infection averages 6 to 7 weeks, with a range of 2 weeks to 6 months ***(Bjoro et al., 1994)***. The clinical picture of disease in children is indistinguishable from hepatitis A- or B-associated disease. Most pediatric patients are asymptomatic. Symptomatic infections usually are mild and insidious in onset. Jaundice occurs in only 25% of patients, and elevations in alanine aminotransferase (ALT) generally are lower than those in hepatitis B virus infection. Fulminant hepatitis occurs but is extremely uncommon. With chronic disease, autoimmune complications are common (e.g., autoimmune hepatitis, arthritis, serum sickness, and erythema multiforme). Children with an underlying immunodeficiency disorder have a higher and more rapid rate of disease progression ***(Alter,1995)***. Hepatocellular carcinoma develops in a small proportion of patients who have chronic hepatitis, but the true rate of this complication is unknown. It is not known whether the risk of chronic disease and subsequent complications is higher for patients infected as newborns than for patients infected at an older age. Persistent infection develops in at least 85% of infected newborns, even in the absence of biochemical evidence of liver disease ***(Alter et al., 1992, Shakil et al., 1995)***. Chronic hepatitis occurs in ~70% of patients and cirrhosis in ~20% ***(Bjoro et al., 1994)***.

VIII-Extrahepatic manifestations of HCV

The hepatitis C virus (HCV) is a cause of both acute and chronic hepatitis. In addition, several extrahepatic diseases have been associated with chronic HCV infection, and in most cases appear to be directly related to the viral infection ***(Gumber et al., 1995, Pawlotsky et al., 1994, Cacoub et al., 2000, El-Serag et al., 2002).***

These include:

- Hematological diseases such as cryoglobulinemia and lymphoma.
- Autoimmune disorders such as thyroiditis and the presence of auto antibodies.
- Renal disease
- Dermatologic conditions such as lichen planus and porphyria cutanea tarda.

HEMATOLOGIC DISORDERS:-

Essential mixed cryoglobulinemia(EMC): Mixed cryoglobulinemia is a lymphoproliferative disorder that can lead to deposition of circulating immune complexes in small to medium sized blood vessels. It often presents with the clinical triad of palpable purpura, arthralgias, and weakness, but can also involve the kidneys, peripheral nerves, and brain.

HCV infection appears to play an etiologic role in most patients with essential mixed cryoglobulinemia. As an example, three studies of 101 patients with this disorder found that 95 (95 percent) had one or more of the following signs of HCV infection ***(Agnello et al 1992; Misiani et al 1992; Pozzato et al 1994)***:

- Circulating anti-HCV antibodies.
- The presence of polyclonal IgG anti-HCV antibodies within the cryoprecipitate.
- HCV RNA in the plasma and particularly the cryoprecipitate.

Another report prospectively evaluated the prevalence of cryoglobulins in 226 patients with chronic liver disease ***(Lunel et al 1994)***. Of the 127 with chronic HCV infection, cryoglobulins were found in 69 (54 percent), frequently with anti-HCV antibody and HCV RNA concentrated in the cryoprecipitates. This incorporation of virus and antibody into the cryoprecipitate may sometimes reduce serum levels of anti-HCV and HCV RNA below detectable levels; this must be kept in mind when attempting to diagnose HCV infection in these patients.

The association of mixed cryoglobulinemia with HCV infection may be linked to the ability of the virus to bind to B lymphocytes via CD81 ***(Pileri et al., 1998)***. Binding lowers the activation threshold of these cells, thereby facilitating the production of autoantibodies.

Further evidence for a causal relationship between HCV and essential mixed cryoglobulinemia is the demonstration of anti-HCV antibodies in the vessel walls of skin biopsies obtained from patients with mixed cryoglobulinemia and cutaneous vasculitis. In addition, cryoglobulin levels decrease and skin lesions and symptoms improve in association with a reduction in HCV virus when patients respond to treatment with interferon alfa ***(Ferri et al., 1993; Lunel et al., 1994)***.

Unfortunately, not all patients with HCV infection and cryoglobulinemia respond to interferon treatment. In addition, a reduction in cryoglobulin titers is not directly associated with a decrease in serum alanine aminotransferase (ALT) or HCV RNA.

Treatment of patients with cryoglobulinemia due to HCV should be based upon the presence of cryoglobulinemia symptoms rather than the usual criteria used in patients with chronic hepatitis alone. Similarly, the response should be assessed by symptomatic improvement of cryoglobulinemia, a reduction in cryocrit, and an increase in serum complement levels. Complete responses may be more common in patients with low pretreatment levels of viremia and with high dose interferon regimens ***(Casato et al., 1997).***

Monoclonal gammopathies: Hepatitis C may be a risk factor for the development of monoclonal gammopathies. Prior to the development of tests for the hepatitis C virus, the prevalence of monoclonal gammopathies was noted to be increased in patients with chronic liver disease ***(Heer et al., 1984).*** The ability to test for the hepatitis C virus permitted identification of patients who may be at greatest risk. A study of 239 HCV-positive patients compared to 98 HCV-negative controls (76 with chronic hepatitis B, 9 with alcoholic liver disease, and 13 with primary biliary cirrhosis) made the following observations ***(Andreone et al., 1998)***:

- Monoclonal bands were detected in 11 percent (compared to 1 percent of a control, age-matched population).
- The incidence peaked in the seventh decade.
- Nine of the 26 patients with a monoclonal band had either a smoldering myeloma or multiple myeloma .
- Monoclonal gammopathy was most often associated with HCV genotype 2a/c.
- **Lymphoma** : will be discussed in details in chapter 3.

• **Diabetes Mellitus:** HCV infection has been linked to diabetes mellitus in several epidemiologic studies ***(Allison et al., 1994;Mehta et al., 2003)***. As an example, a retrospective analysis of 1117 patients with chronic viral hepatitis found that diabetes was present in significantly more patients with hepatitis C compared to hepatitis B virus (HBV) infection (21 versus 12 percent) ***(Mason et al., 1999)***. HCV genotype 2a was overrepresented among the diabetic patients. In a separate case-control trial included in the same report, the prevalence of HCV infection was significantly higher among patients with diabetes mellitus compared to controls (4.2 versus 1.6 percent) ***(Shintani et al., 2004)***.

Risk factors for the development of diabetes mellitus in HCV infected patients included older age, obesity, severe liver fibrosis, and a family history of diabetes mellitus ***(Petit et al., 2001)***. Patients undergoing liver transplantation for HCV also appear to be at increased risk, compared to other liver diseases, for developing diabetes mellitus following transplantation ***(Bigam et al., 2000)***.

The cause of these associations is unknown, but their magnitude may be overestimated based upon the retrospective nature of the above reports and the following factors ***(Hadziyannis et al., 1999)***:

- Patients with diabetes have more parenteral exposures than the general population, placing them at increased risk for transmission of viruses.
- Not all studies are controlled for the presence of cirrhosis, which may be associated with impaired glucose tolerance.

- HCV has also been linked to insulin resistance without overt diabetes. It has been suggested that the associated insulin resistance may contribute to fibrosis progression ***(Hui et al., 2003)***.

AUTOIMMUNE DISORDERS — A number of autoimmune disorders have been associated with HCV infection, including autoantibody formation, thyroid disease, sialoadenitis, and autoimmune idiopathic thrombocytopenic purpura.

Autoantibodies — Autoantibodies are common in patients with chronic HCV infection; antinuclear antibodies, antibodies directed against the Fc portion of IgG (rheumatoid factor), anticardiolipin antibodies, smooth muscle antibodies, or antithyroid antibodies are detected in 40 to 65 percent of patients ***(Clifford et al., 1995; Cacoub et al., 1997; Cacoub et al., 2000)***. These antibodies are typically present in low titer, and do not appear to influence the presentation or course of infection; they are not usually associated with extrahepatic disease.

However, their presence may result in diagnostic difficulties; as an example, the HCV-infected patient with arthralgias, arthritis, and rheumatoid factor positivity may be initially misdiagnosed as having rheumatoid arthritis. In this setting, testing for other RA-associated autoantibodies infrequently observed in patients with HCV infection, such as antikeratin antibodies, may be helpful diagnostically ***(Kessel et al., 2000)***.

Antibodies to actin and to liver/kidney microsomes (anti-LKM-1) are characteristic of types 1 and 2 autoimmune hepatitis, respectively. These antibodies have been detected in some patients with chronic HCV infection, particularly in Europe ***(Reddy et al., 1995; Zauli et al., 1997)***.

Most patients with hepatitis C and anti-LKM-1 antibodies, as well as other types of non-organ-specific autoantibodies, appear to benefit from interferon to the same extent as patients with chronic hepatitis C without such antibodies. However, such patients need meticulous monitoring

during interferon treatment, since flares of aminotransferases without subsequent clearance of HCV RNA have been observed ***(Muratori et al., 1994; Gschwantler et al., 1995)***. This observation suggests that these patients may behave as if they had autoimmune hepatitis. In support of this hypothesis is the finding that when patients with chronic hepatitis C with or without anti-LKM-1 antibodies were compared, the viral load was lower in the patients with anti-LKM-1 antibodies even though both groups had disease of similar severity ***(Giostra et al., 1996)***. Furthermore, some of these patients have responded to prednisone and azathioprine, directed against presumed autoimmune hepatitis ***(Bortolotti et al., 1996; Bellary et al., 1995)***. One possible method of determining whether the hepatitis is primarily due to HCV or autoimmune hepatitis is that the anti-LKM-1 antibodies in patients with HCV are directed at different epitopes of cytochrome P450 2D6 (CYP2D6, the target antigen) from that seen with autoimmune hepatitis ***(Yamamoto et al., 1993; Muratori et al., 1994)***.

Thyroid disease — Thyroid disorders are common in patients with chronic HCV, particularly women ***(Tran et al., 1993; Antonelli et al., 2004)***.

All patients receiving interferon alfa should be monitored for thyroid disease, particularly women and patients with preexisting antithyroid antibodies. Interferon therapy usually can be continued while hypothyroidism is being treated. On the other hand, we have usually stopped interferon in patients who develop clinically apparent hyperthyroidism ***(Roti et al., 1996; Marazuela et al., 1996; Deutsch et al., 1997; Antonelli et al., 2004)***.

Sialoadenitis — A lymphocytic sialadenitis suggestive of Sjogren's syndrome has been described in patients with chronic HCV infection. In one series of 28 HCV-infected patients and 20 controls, for example, these

histologic changes were significantly more common in the labial salivary glands of the HCV-infected patients (57 versus 5 %) ***(Haddad et al., 1992)***.

Autoimmune idiopathic thrombocytopenic purpura — Anti-HCV antibodies occur in 10 to 19 percent of patients with autoimmune idiopathic thrombocytopenic purpura (ITP). However, the diagnosis of autoimmune ITP usually predates HCV infection, suggesting that the latter results from the transfusion of blood products ***(Pawlotsky et al., 1995)***. On the other hand, ITP has been reported to develop during interferon therapy for HCV. Thus, the relationship between autoimmune ITP and HCV remains to be clarified.

Myasthenia gravis: An association between myasthenia gravis (MG) and hepatitis C virus infection has been suggested in case reports ***(Reading et al., 1998; Eddy et al., 1999)***, although a causal association has not been clearly established ***(Halfon et al., 1996)***. MG has also been

described in association with administration of interferon, possibly because of exacerbation of preexisting subclinical disease ***(Harada et al., 1999; Gurtubay et al., 1999)***.

OCULAR DISEASE — HCV infection has been associated with a variety ophthalmologic disorders including corneal ulcers (Mooren's ulcer), uveitis, and scleritis ***(Wilson et al., 1993; Moder et al., 2000)***, and sicca syndrome in patients with HCV-related Sjogren's syndrome ***(Ramos-Casals et al., 1999)***. In addition, ophthalmologic disorders (retinal hemorrhages, cotton wool spots, and rarely retinal artery or vein obstruction) can occur during interferon therapy.

RENAL DISEASE — Glomerular disease may occur in patients with chronic HCV infection. The most common patterns are membranoproliferative glomerulonephritis (usually associated with essential mixed cryoglobulinemia), (due to deposition of immune complexes containing anti-HCV and HCV RNA in the glomeruli) and, less frequently, membranous nephropathy ***(Johnson et al., 1994)***.

Interferon alfa is indicated in patients with mixed cryoglobulinemia and membranoproliferative glomerulonephritis. A number of studies have reported a beneficial response to antiviral therapy in this setting, and the reduction in proteinuria correlates with a fall in HCV RNA ***(Agnello et al., 1992; Johnson et al., 1993; Johnson et al., 1994)***. However, long-term responses to interferon are unusual; maintenance treatment may be required, and renal function is often not improved by treatment.

DERMATOLOGIC DISEASE — A variety of dermatologic diseases may be associated with HCV infection ***(Daoud et al., 1995)***.

Porphyria cutanea tarda — (PCT) is a skin disease caused by a reduction of hepatic uroporphyrinogen decarboxylase activity that is characterized by photosensitivity, skin fragility, bruising, and vesicles or bullae that can become hemorrhagic. There is a strong association between the sporadic form of PCT and HCV infection. The precise mechanism by which HCV infection might cause or act as a trigger for PCT in predisposed subjects is not known ***(Daoud et al., 1995)***.

Leukocytoclastic vasculitis — A leukocytoclastic vasculitis may occur in conjunction with essential mixed cryoglobulinemia, presenting clinically with palpable purpura and petechiae that usually involve the lower extremities . Skin biopsy demonstrates cutaneous vasculitis with dermal blood vessel destruction associated with neutrophilic infiltration in

and around the vessel wall.Other tissues, particularly the lower extremity peripheral nerves, may show similar vasculitic changes involving the vasa nervosum ***(David et al., 1996)***.This may be manifested clinically as a peripheral neuropathy which, as in other forms of vasculitis, is typically asymmetric (also called a mononeuritis multiplex).

Lichen planus — Lichen planus (LP) is characterized by flat-topped, violaceous, pruritic papules with a generalized distribution. It can also involve mucus membranes, hair, and nails. LP may be mediated through the cellular immune response, although the actual precipitating mechanism is not known ***(Pilli et al., 2002)***. Skin biopsy demonstrates a dense lymphocytic infiltration in the upper dermis.

BONE DISEASE — Hepatitis C-associated osteosclerosis is a rare disorder characterized by a marked increase in bone mass during adult life. While most cases have been reported in patients with a history of intravenous drug abuse, it has also been seen with hepatitis C after blood transfusion ***(Shaker et al., 1999)***. Periosteal, endosteal and trabecular bone thickening occurs throughout the skeleton with the exception of the cranium. During active disease, forearm and leg pain are common, bone remodeling (turnover) is high, and bone mineral density is two- to three-fold higher than age-matched norms. Abnormalities in insulin-like growth factors (IGF-1 and IGF-II) or their binding proteins may contribute to the increase in bone formation in this disorder ***(Khosla et al., 1998)***.

IX- Diagnostic methods of HCV infection

Since the discovery of HCV in 1989 using molecular biology techniques, there was a rapid evaluation in the field of hepatitis-C diagnostic methods. The current ability to diagnose and manage chronic hepatitis C is almost entirely the result of applied biotechnology in addition to assaying for serum antibodies against viral proteins, serum and liver tissue can be tested for viral RNA and for evidence of ongoing viral replication ***(Leniewski et al., 1995)***.

1- Screening assays for anti-HCV:

The main screening test for detecting anti-HCV is the enzyme immuno assay (EIA). The EIA has many advantages in the diagnostic setting including ease of use, low variability and relatively low expense ***(Younossi and Mchutchison, 1996)***.

These tests results are also affected by the immune status of the host, and they may give false negative results in patients with illness that affect their serologic responses ***(Pereira and Levey, 1997)***.

The first generation anti-HCV test (EIA-1) contained a single HCV recombinant antigen derived from the non-structural Ns4 gene, designated $C_{100\text{-}3}$, this antigen represents 363 amino acids of viral Ns4 sequence from the Ns4 region ***(Gretch et al., 1992)***.

Although development of EIA-1 represented a break through in terms of identifying patients with serologic evidence of HCV infection EIA-1 lacked optimal sensitivity and specificity ***(Gretch et al., 1992)***.

Subsequently, the EIA-1 test was replaced in 1992 by the second-generation test (EIA-2). EIA-2 test contains HCV antigen from the core and Ns3 genes in addition to the Ns4-derived antigen and thus represents a multiantigen EIA. This leads to improvement in sensitivity and slight increase in specificity relative to EIA-1 ***(Kleinman et al., 1992, Lok et al., 1993 and Costa et al., 1999)***.

A third generation anti-HCV test (EIA-3) is being used for screening assay. This EIA-3 test contains reconfigured core and Ns3 antigens plus an additional HCV antigen Ns5 that is not present in EIA-2 test. This led to incremental improvement in sensitivity for detecting HCV infection ***(Haflon et al., 1998)***.

However, testing in high prevalence population has indicated that not all patients with active HCV infection e.g (HCV-RNA positive) are identified with EIA screening tests ***(Leniewski et al., 1995)***. Moreover, the currently available EIA-3 tests are claimed for lacking of sensitivity in seroconversions due to infection with HCV-genotypes 2 and 3a ***(Martens et al., 1999)***.

2-Supplemental tests for anti-HCV:-

Supplemental tests for anti-HCV were developed to help to resolve false-positive EIA tests results.

First generation supplementary tests Recombinant ImmunBlot Assay (RIBA-1) have used recombinant antigen from Ns4, Ns3, and Ns5. They had been improved by the addition of two extra protein bands to make the second generation RIBA-2 which permits the detection of antibodies to individual recombinant HCV-antigens: C22, C33, C100 ***(Younossi and McHutchison, 1996)***.

Patients who react to two or more HCV antigen are considered to be RIBA positive where those who react to one antigen only are considered to have intermediate results ***(Younossi and McHutchison, 1996)***.

In 1999, the FDA licensed an anti-HCV confirmatory assay, (RIBA) 3.0., there are synthetic peptides from the core and NS4 regions and recombinant antigens from NS3 and NS5-derived C33 antigen enhanced the sensitivity of the RIBA-3 system compared with previous generations ***(Damen et al., 1995)*** and reduced the number of intermediate results ***(Pawlotsky et al., 1996)***.

3-Molecular investigations:-

Confirmation of diagnosis of ongoing HCV infection relies on the detection of viremia. HCV-RNA testing requires an amplification

technique and thus sensitive assays were developed based on polymerase chain reaction (PCR). Numerous factors contribute to PCR assay variability including specimen handling and storage conditions, correct design and variability of biochemical reaction as well as DNA product contaminations ***(Young et al., 1995)***.

A-QUALITATIVE TESTS FOR HCV-RNA:-

Detection of HCV RNA in serum by highly sensitive tests such as reverse transcription PCR (RT-PCR) became an increasingly important tool for confirming the diagnosis of hepatitis-C and for assessing the virological response to therapy ***(Zaaijer et al., 1993; Goncales et al., 1998)***.

Collectively detection of HCV-RNA by PCR can be used for:-

1. Confirmation of HCV infection in patients with RIBA positive or intermediate anti-HCV antibody results ***(Farci et al., 1991)***.
2. Early diagnosis in patients with acute hepatitis as viral RNA appear much earlier than other markers within days of infection ***(Farci et al., 1991)***.
3. Detection of HCV-RNA may confirm HCV viremia in immunocompromised patients in whom antibody responses may be impaired .
4. Monitoring of perinatal transmission of HCV from chronically infected mothers ***(Thaler et al., 1990).***
5. Study of HCV-RNA dynamics during the course of therapy ***(Torre et al., 1999)***.

B-QUANTITATIVE TESTS FOR HCV-RNA:

Quantitative assays ascertain the quantity of HCV RNA in blood using either target amplification or signal amplification techniques (branched DNA assay). The level of HCV RNA in blood helps in predicting the likelihood of response to treatment, and the change in the level of HCV RNA during treatment can be used to monitor response. The results should be reported in international units to standardize data ***(Saldanha et al., 1999)*** although the dynamic ranges differ and the results can be difficult to compare between assays. Because a change in the HCV RNA level is used to monitor treatment response, it is important at the outset of treatment to obtain the actual level rather than simply a report indicating that the level exceeds an upper limit of detection, since HCV RNA levels sometimes are above the linear range of currently available assays. In addition, the same quantitative test should be used while on therapy to avoid confusion. The only quantitative test that has currently

received FDA approval is VERSANT® HCV RNA version 3.0 (Bayer Diagnostics, Tarrytown, NY).

Real time quantitative PCR:

RQ-PCR permits accurate quantitation of PCR products during the exponential phase of the PCR amplification process, which is in full contrast to the classical PCR end point quantitation. Owing to the real-time detection of fluorescent signals during and/or after each subsequent PCR cycle, quantitative PCR data can be obtained in a short period of time and no post-PCR processing is needed. At present, three main types of RQ PCR techniques are available:-

I- Using SYBR Green I Dye ***(Ritie et al., 1997)***.

II- Using hydrolysis probes ***(Kreuzer et al., 2001)***.

III- Using hybridization probes ***(VanDongen et al., 2003)***.

4-HCV Ag detection:

Since antibody tests are unable to identify subjects in the early stage of infection, in what is known as the diagnostic window period, during which specific antibodies have not yet been produced, but the virus is present in the plasma, sometimes in large quantities. This stage prior to seroconversion may last up to 2 months in immunocompetent subjects and as long as 6 to 12 months in immunodeficient patients ***(Vanderpoel, 1994).***

In the spring of 1999, HCV minipool nucleic acid testing **(MP-NAT)**, which detects HCV RNA rather than later-appearing antibodies, was added to routine blood donor screening ***(Tabor et al.,2002).*** HCV MP-NAT has been estimated to reduce the undetectable infectious window period to approximately 8 to 10 days compared to the 70 day window using

HCV EIA 3.0 antibody testing ***(Schuttler et al., 2000; Busch et al., 2003; Stramer et al., 2004; Hitzler et al., 2004).***

Furthermore, the risk of false positive (for nucleic acid amplification methods) should be considered, and some studies reported a significant percentage of false positive results connected with environmental contamination and carryover ***(Aslonzadeh et al., 1996)***.

Furthermore it is expensive reagents and long execution times, aspects that have a significant effect on the final cost of each test, which is around $50 test when the test is performed in diagnostic laboratories. The cost of NAT based screening of blood donations decreases when tests are performed in pools. Therefore a quick, inexpensive, sensitive, and specific test is clearly needed to identify potentially infective blood units that have not been identified by specific antibody tests ***(Iceardi et al., 2001)***.

A new test has recently been developed to detect the HCV core protein **(HCV antigen [Ag])**, which is coded for by one of the most conserved regions of the (N-terminal region of the core protein), virus genome and in which anti-HCV-positive patients appears to be correlated with HCV RNA level ***(Komatsu and Takasaki, 1999)***. This protein may be an ideal target for the development of methods to identify samples from individual in the early stage of infection ***(Peterson et al., 2000)***.

5-HCV Genotyping:-

HCV genotyping provides important informations in epidemiological studies but does not help in confirming the diagnosis of HCV infection ***(Margin et al., 1996)***.

Test to determine HCV genotype fall into two general categories:-

1. Screening tests that detect point mutations within the HCV genome.
2. Confirmatory tests that evaluate larger segments of HCV genes. Gold standard confirmatory test for HCV genotype determination include nucleotide sequencing and phylogenetic analysis of the E_1 gene or Ns_5B gene ***(Stuyver et al., 1994)***.

HCV genotyping is an important aspect of the ongoing clinical trials, as regards correlation to disease severity and progression ***(Lindsay et al., 1996)***.

6-Serotypes:

New stereotyping assays, based on homologus reaction between type specific antibody in the serum and peptide bound to a solid phase, can detect all six major types by using peptides derived from Ns4 region ***(Dusheiko et al., 1996)***. It should be stressed that the same individual can be simultaneously or successively infected by several types of HCV, which is frequent in hemophiliacs ***(Rumi et al., 1990)***.

7- Biochemical investigations:

Much study is continuing to assess the use of serum markers in predicting progress, severity and response to treatment of chronic hepatitis C. Some data demonstrated that metabolic changes, like glucose intolerance and hyperlipidemia, are correlated to the different stages of liver disease and could negatively affect the evaluation of hepatitis ***(Cimino et al., 2000)***.

Markers of oxidative stress have been reported to reflect the degree of liver damage in HCV infected patient of which 8-hydroxyguanosine in leucocytes and urinary 8-isoprostan are under more studies ***(Gardin et al., 1999)***.

It has been suggested that advanced liver disease may favor the appearance of serum cryoglobulins without clinical relevance, while the vasculitic syndrome, with IgM monoclonal rheumatoid factor protects against the progression of liver damage ***(Lenzi et al., 1998)***.

8-Histopathology:

Liver biopsy is often employed to determine the initial level of inflammation and hepatocellular damage and to monitor evaluation of disease in HCV-infected individuals ***(Perrillo, 1997)***.

The degree of inflammation seems to correlate with the circulating level of HCV RNA ***(Gordon et al., 1994)***.

Five histopatological features have been observed to be relatively characteristic of although not pathogenomic for, chronic HCV infection:-

1- Lymphoid aggregates in portal tracts.
2- Degenerative changes of bile duct.
3- Large droplet steatosis.
4- Mallory body-like material with in injured hepatocytes.
5- Lymphocytic aggregation within the lobules ***(Bach et al., 1998)***.

The two most striking and characteristic finding, however, are bile duct damage and portal tract based lymphoid aggregates ***(Lefkowitch et al., 1993)***.

X- Treatment options of HCV in children

1-Diet and lifestyle

It is possible that a diet with an excess of calories, fat, carbohydrates, and iron may adversely affect the long-term prognosis of HCV-infected children; however, this has yet to be established in clinical investigations. At this point, it seems most prudent to recommend a well-balanced diet with attempts at weight loss for significantly overweight children, but restriction of calories, protein, and iron in generally healthy children is not warranted ***(Hickman et al., 2004).***

Children and adolescents infected with hepatitis C should be immunized against hepatitis A and hepatitis B to prevent additional liver damage. Pneumococcal and influenza vaccines should be offered if appropriate ***(Schwimmer et al., 2000).***

Support should be provided to prevent social stigmatization of children with HCV infection. The children and their families should be well educated about the nature of this condition, as well as its modes of transmission. Children with HCV should not be restricted in their daily activities nor excluded from day care centers, schools, or sports. Standard precautions should be used in situations where exposure to blood occurs ***(Seeff et al., 2002)***. Sharing of razors, toothbrushes, pierced earrings, and nail clippers should be discouraged.

2-Pharmacologic treatment:

A crucial step in the treatment of HCV infection in children involves identifying appropriate candidates for therapy based on careful assessment of risks and benefits of the intervention ***(Jonas et al., 2002)***.

The best indicator of effective treatment is sustained viral response (SVR), defined by the absence of detectable HCV RNA in the serum at 24 weeks after the end of treatment.

Sustained response to therapy is lower in children with HCV genotype 1 as compared to other genotypes ***(Gonzalez et al., 2002)***.

Combination therapy consisting of interferon-alfa 2b and ribavirin was recently approved in the United States by the FDA for use in children 3 years of age and older. SVR of 67% has been reported with this therapy in children ***(Gonzalez et al., 2002)*** . No other therapy has been approved.

Limited data are available about the pharmacokinetics of peginterferon-alfa 2a in children ***(Schwarz et al., 2003).*** A large multicenter trial of this product is planned.

A)Antivirals

- ***Interferon alfa-2b***

Standard dosage : 3 million IU/m2 of body surface area subcutaneously three times per week. Adult dosing should be used for individuals who weigh more than 61 kg. Duration of therapy should be 48 weeks for genotype 1 and 24 weeks for genotypes 2 or 3. Genotype 4 infections should probably be treated for 48 weeks, but data are more limited. In genotype 1 infections, lack of viral clearance (by polymerase

chain reaction) at 24 weeks indicates nonresponse, and therapy should be discontinued ***(Jacobson et al., 2002).***

Contraindications: Hypersensitivity to any interferon-alfa preparations, autoimmune hepatitis, decompensated liver disease, pregnancy, and solid organ transplant other than liver, May aggravate depression, neuropsychiatric, autoimmune, ischemic, ophthalmologic, and infectious disorders. Use caution if seizure disorder or central nervous system

(CNS) disorder, cardiac disease, severe liver disease, renal disease, diabetes, thyroid disorder, history of pulmonary disease, or hypertension is present ***(Figlerowicz et al., 2004)***.

Main drug interactions : Bone marrow suppressants (alefacept, ibritumomab tiuxetan, and rituximab), colchicine, zidovudine. Avoid theophyllines as combination may increase theophylline levels and lead to toxicity. Use live vaccines more than two weeks before or more than three months after immunosuppressive therapy; otherwise, coadministration may result in inadequate immunologic response to vaccines and increase the risk of disseminated infection ***(Figlerowicz et al., 2004).***

Main side effects : Adverse events commonly reported in children include influenza-like syndrome, hair loss, weight loss, and neutropenia, all of which were reversible and rarely required withdrawal of interferon ***(Jacobson et al.,2002; Figlerowicz et al.,2004)***. Suicidal ideation has been noted to occur more frequently among pediatric patients compared to adult patients during treatment and off-therapy follow-up ***(Rebetron et al., 2002)***.

Other reported side effects are similar to those in adults, but evidence thus far demonstrates that children tolerate interferon better than adults

(Hardikar et al., 2002). Common adverse reactions reported in adults include flu-like symptoms, rash, gastrointestinal symptoms (anorexia, nausea, vomiting, abdominal pain, diarrhea), depression, emotional lability, impaired thought, anxiety, arthralgia, dry mouth, throat irritation, cough, dizziness, headache, paresthesias, lymphadenopathy, and reactions at the injection site. Serious reactions reported in adults include anaphylaxis (rare), aplastic anemia (rare), autoimmune diseases (rare), seizures, hepatotoxicity, pulmonary toxicity, nephrotic syndrome and renal failure, pancreatitis, suicidal ideation, delirium, peripheral neuropathy, arrhythmias, cardiomyopathy, myocardial infarction, gastrointestinal bleed, hypertension, and retinal hemorrhages *(Hardikar et al., 2002)*.

Special points:Complete blood count should be measured at baseline and should be monitored one and two weeks after initiation of therapy and then monthly thereafter. ALT should be measured at 3-month intervals. Electrolytes and thyroid-stimulating hormone should be assessed at baseline and periodically during course of therapy.

Dose should be reduced by 50% or medication discontinued if severe adverse reactions develop. Initial dose may be resumed at 100% once adverse reactions have abated or laboratory abnormalities have returned to normal *(Rebetron et al.,2002)*.

- ***Ribavirin***

Standard dosage 15 mg/kg/day divided into 2 dosages. Adult dosing may be used if patient's weight is greater than 61 kg (for weight 75 kg or less use 400 mg every morning and 600 mg every evening; for weight greater than 75 kg use 600 mg twice a day). Available as 200-mg capsules or 40-mg/mL oral solution *(Jacobson et al., 2002)*.

Contraindications: Hypersensitivity to ribavirin, pregnancy, hemoglobinopathies (*ie*, thalassemia major, sickle cell anemia), unstable cardiac disease, creatinine clearance greater than 50 mL/min. Use caution with cardiac disease or myelosuppression ***(Figlerowicz et al., 2004).***

Main drug interactions: Antiviral agents used to treat HIV infection (abacavir, didanosine, emtricitabine, lamivudine, stavudine, zalcitabine, zidovudine) as combination with any of these agents may increase the risk of lactic acidosis. Combination with zidovudine may also decrease the efficacy of zidovudine ***(Rebetron et al., 2002).***

Main side effects: Common reactions reported in adults include fatigue, headache, anorexia, dyspepsia, nausea, rash, pruritus, conjunctivitis, cough, and headache. May cause sun sensitivity. Serious adverse reactions reported in adults include hemolytic anemia, pancreatitis, worsening respiratory status that may be life threatening, cardiac arrest, hypotension, bradycardia, and teratogenicity / embryocidal effects. ***(Figlerowicz et al., 2004).***

Special points : Should be taken with food. Half-life is 12 days and persists in nonplasma compartments for as long as 6 months. Given the significant embryocidal and/or teratogenic effects of this drug, pregnancy should be avoided during therapy and for 6 months after completion of therapy in both female patients and female partners of male patients taking ribavirin. Females of childbearing potential should undergo pregnancy tests at baseline (prior to initiation of ribavirin) and then monthly during therapy. Complete blood count should be monitored at week 0, 2, and 4 of therapy and then periodically. Approved for treatment of HCV infection in children only in combination with interferon alfa-2b ***(Rebetron et al., 2002).***

3-Emerging therapies

A) Alternative interferon and ribavirin-like drugs

A number of modifications of IFN have been examined to increase its bioavailability and optimize its pharmacokinetics. Pegylated interferon has essentially replaced standard interferon in adults treated for HCV infection ***(Manns et al., 2001)***.

Comparisons of peginterferon alfa plus ribavirin with interferon alfa-2b plus ribavirin have demonstrated higher SVR rates for the former. In addition, the pegylated forms of interferon offer other advantages including fewer side effects and greater ease of administration (injections once weekly rather than three times per week) ***(Manns et al., 2001)***. Other modifications to interferon are being evaluated, including conjugation with albumin, liposome encapsulation, polyamino acid-based oral delivering systems, and others. Agents similar to ribavirin but with fewer side effects are also being explored. Examples include viramidine (liver-targeting prodrug of ribavirin) and levovirin (L-isomer of ribavirin) which have been well tolerated in animal studies and in phase I trials ***(Hugle et al., 2003)***.

B) Protease inhibitors and polymerase inhibitors

The use of protease inhibitors is a promising molecular strategy for the treatment of HCV infection. BILN 2061 (oral) has been well tolerated in phase I trials in which it demonstrated good virologic results, and it is being evaluated in phase II trials. VX-950 is another protease inhibitor under early investigation. JTK-003 is a polymerase inhibitor currently under investigation in phase I and II trials ***(Hugle et al., 2003)***.

C) Ribozymes, antisense oligodeoxynucleotide, and small interfering RNAs

Ribozymes targeting HCV RNA have been developed. Hepatozyme is a nuclease-stabilized ribozyme with potent antiviral activity in cell culture.

However, in vivo studies have not led to the expected clinically significant effects and demonstrated important adverse effects and intolerance. Antisense oligodeoxynucleotides target HCV RNA and inhibit the expression of HCV proteins. The use of antisense oligodeoxynucleotides such as ISIS 14803 in the treatment of hepatitis C is under early investigation. Small interfering RNAs (SiRNAs) constitute a recently discovered system that may be considered endogenous antisense RNA molecules and may lead to promising therapies in the future ***(Hugle et al., 2003)***.

D) Antibodies

Monoclonal antibodies (mAbs) against several HCV proteins including envelope protein (E2) among others are currently under investigation. Early studies are showing promising results. An important advantage of mAbs is their specificity. However, the action and use of mAbs may be limited, as they are not expected to be useful in targeting replication, which is an intracellular process. Monoclonal antibodies may be particularly useful to treat cases of HCV exposure or in HCV-infected liver transplant recipients by providing protection against circulating HCV ***(Hugle et al., 2003)***.

E) Immunomodulation

Thymosin alpha-1, a synthetic polypeptide derived from thymus gland extracts, promotes T-cell maturation and natural killer cell activity. In addition, it appears to stimulate the production of IFN gamma, interleukin (IL)-2 and IL-3 ***(Hugle et al., 2003)***.

The combined use of thymosin alpha-1 and interferon may lead to higher response than current therapies. Multiple clinical trials are presently investigating the role of thymosin alpha-1 in the treatment of HCV infection in adults. Inosine monophosphate dehydrogenase (IMPDH) is an enzyme responsible for catalyzing an essential step of the de novo biosynthesis of guanosine nucleotides. Inhibition of IMPDH leads to a series of antiviral and anti-inflammatory activities that are not fully understood, and may represent an alternative to ribavirin. Mycophenylate mofetil, another IMPDH inhibitor, is being evaluated in combination with pegylated IFN for the treatment of HCV infection in adults ***(Hugle et al., 2003)***.

Histamine dihydrochloride is being studied in combination with interferon and ribavirin. Histamine dihydrochloride can activate natural killer cells or T-lymphocytes. Amantadine, an antiviral agent used against influenza A infection, is also being evaluated as adjunct therapy for HCV although data remains controversial. Tumor necrosis factor (TNF) may exacerbate resistance to interferon. Therefore, use of antagonists to TNF is a potentially reasonable adjunct therapy. Early trials have demonstrated promising results although additional longer trials are still needed to establish the role of anti- TNF in the treatment of HCV ***(Hugle et al., 2003)***.

F) Therapeutic vaccination

Effective immunization to prevent HCV infection has not been possible given the high genetic variability of HCV. However, the potential role of a therapeutic vaccine is currently under investigation and may constitute a more realistic objective to achieve ***(Hugle et al., 2003)***.

Non-Hodgkin Lymphoma in Children

Lymphoma is the third most common childhood cancer after brain tumors and leukemia. Lymphomas are divided into two main types. The first is called Hodgkin's lymphoma or Hodgkin's disease, which is named after Dr. Thomas Hodgkin, who first diagnosed it in 1832. All other types of lymphoma are called non-Hodgkin lymphoma(NHL). These two types of lymphomas differ in their behavior, pathology, spread, and responsiveness to treatment ***(American Cancer Society, 2005).***

Lymphoma is a type of cancer that starts in lymphoid tissue. Other types of cancer can develop in other organs of children and adults and then spread to lymphoid tissue. For example, childhood cancers such as neuroblastoma or Wilms' tumor can spread to lymph nodes, but these cancers are not lymphomas ***(Ries et al., 1999).***

The main cell type found in lymphoid tissue is the lymphocyte, a type of white blood cell. The two main types of lymphocytes are B lymphocytes and T lymphocytes. Although both types of cells can develop into lymphomas, B-cell lymphomas are more common than T-cell lymphomas ***(Sandlund et al., 2004).***

Normal B cells and T cells can be recognized by laboratory tests that identify distinctive substances on their surfaces. Certain substances are found only on B cells, and others are found only on T cells. There are also several stages of B-cell and T-cell development (maturation) that can be recognized ***(Weinstein et al., 2001)***.

Organs That Contain Lymphoid Tissue

1-Lymph nodes. 2-The thymus gland .

3-The spleen is the largest collection of lymph tissue in the body.

4-Adenoids and tonsils . 5-The stomach and intestinal tract .

6-Bone marrow.

INCIDENCE AND EPIDEMIOLOGY

Incidence

NHL accounts for 5-7 % of malignant diseases in childhood (Europe, United States). It is third common childhood malignancy with an overall incidence of 10.5 per million. The incidence of NHL is higher in the Middle East, Nigeria, and Uganda (15 of 100,000 children under 5-10 years of age) as a result of their increased incidence of endemic Burkitt's lymphoma. NHL accounts for 45 % of all childhood lymphomas in children and adolescents less than 20 years of age. There has been a stable incidence for children less than 15 years of age during the last two decades, but in adolescents 15-19 years of age there has been an increase of 50% to 16.3 per million in 1990-1995. Isolated cases of familial NHL occur ***(Lanzkowsky, 2005).***

Epidemiology

- Sex ratio of NHL is 2.5 male: 1 female with peak age 5-15 years.

Risk factors:

1-Immunodeficiency:

Immunodeficiency, including both congenital and acquired conditions, is the strongest risk factor known to increase NHL risk ***(Rabkin et al., 1997)***. NHL is the most frequent malignancy in young persons with ataxia telangiectasia or the Wiskott-Aldrich syndrome, as well as in children with X-linked lymphoproliferative syndrome or combined immunodeficiency ***(Filipovich et al., 1992)***.

However, these congenital immunodeficiency disorders are rare and thus have not contributed to the increasing incidence of NHL.

Patients who are treated with immunosuppressive drugs following solid organ or bone marrow transplantation are at substantially increased risk (30 to 50 times) for NHL ***(Hoover et al., 1992)***. Chronic antigenic stimulation induced by the graft and the accompanying immunosuppression are probable mechanisms ***(Scherr et al., 1996)***.

Polyclonal B-cell proliferation is often seen first in transplant patients, and is occasionally reversible when the immunosuppressive therapy is stopped. It may persist and evolve into a monoclonal disease. Loss of control of persistent Ebstien Barr Virus (EBV) infection caused by the immunosuppressive therapy appears to be part of this process ***(Scherr et al., 1996)***. However, in spite of the significant increase of organ transplants in the United States in the past decade, these transplant-related

NHL are relatively uncommon and explain very little of the NHL increase in the general population ***(Groves et al., 2000)***.

The risk of NHL in persons infected with HIV is more than 100-fold that of the general population ***(Beral et al., 1991)***. The incidence of NHL increased 20-fold between 1973-1979 and 1988-1990 in the city of San Francisco, in which approximately one-fourth of all single young men were infected with HIV-1 as of 1984 ***(Rabkin et al., 1997)***. These lymphomas are typically of B-cell origin with high-grade histology, are primarily diffuse large cell or Burkitt's lymphoma, and frequently occur in extranodal sites, such as the brain ***(Scherr et al., 1996;Rabkin et al., 1997)***.

However, except possibly in San Francisco, AIDS accounts for a relatively small fraction of NHL ***(Hartge et al., 1992)***. Even with San Francisco excluded from the US data, though, there still is an increased incidence of 2.6% per year in males and 2.0% per year in females ***(Palackdharry et al., 1994)***.

An excess risk of developing NHL has also been reported among patients with rheumatoid arthritis, Sjögren's syndrome, systemic lupus erythematosus, and celiac disease ***(Scherr et al., 1996)***. The estimated risks were lower in populationbased studies ***(Tavani et al., 2000)***. These conditions are also unlikely to contribute dramatically to rising secular trends in NHL because these diseases are rare ***(Groves et al., 2000)***.

2-Infectious organisms other than HIV:

Human T-cell lymphotrophic virus type I (HTLV-I), EBV, Helicobacter pylori, and possibly hepatitis C virus (HCV), have been postulated to play a role in the development of NHL because infection can result in immune system stimulation or dysregulation.

Human T-cell lymphotrophic virus (HTLV), types I and II. HTLV-I is a retrovirus that is endemic in southern Japan and the Caribbean basin ***(Rabkin et al., 1997)***. Infection with HTLV-I, especially in early childhood, is strongly related to adult T-cell leukemia/lymphoma ***(Cleghorn et al., 1995)***. The cumulative lifetime risk for development of adult T-cell leukemia/ lymphoma in infected individuals is estimated to be approximately 5% ***(Mueller et al., 1992)***. Because HTLV-I has a unique geographic distribution, is a relatively rare infection, and causes a specific and rare subtype of NHL, HTLV-I infection is unlikely to account for much of the current increase in NHL ***(Palackdharry et al., 1994; Cleghorn et al., 1995)***.

Epstein-Barr virus. (EBV) appears to be an important co-factor. In addition, host defects in immunoregulation resulting in an imbalance of cytokine production and genetic defects resulting in imprecise and/ or ineffective rearrangement of immunoglobulin and Tcell receptor genes during lymphopoiesis likely contribute to the development of NHL ***(Filipovich et al., 1992)***.

Unlike HTLV-I, EBV infection is a highly prevalent infection in the adult population, with approximately 90% of individuals in developed countries having evidence of previous infection by age 40 ***(Rabkin et al., 1997)***. The association between EBV and lymphoma is well described for both Burkitt's lymphoma and immunodeficiency- associated lymphoma. Nearly all cases of the endemic Burkitt's lymphoma (in Africa and New Guinea) can be shown to contain EBV viral genomic DNA, but the frequency is less than 20% in sporadic cases (in the United States and other developed countries) ***(Rabkin et al., 1997;Palackdharry et al., 1994)***.

Helicobacter pylori. Chronic gastric infection with H. pylori has been linked to the development of lowgrade, mucosa-associated lymphoid tissue (MALT) lymphoma in the stomach ***(Wotherspoonet al., 1998)*** Seropositivity to H. pylori was associated with a 6-fold increased risk of gastric lymphoma in a prospective study ***(Parsonnet et al., 1994)***. These findings suggest that chronic antigenic stimulation and/or inflammation may be important in the development of MALT lymphoma, and perhaps other forms of NHL as well ***(Rabkin et al., 1997)***. However, the declining prevalence of H. pylori infection in the United States suggests that this infectious organism is unlikely to play an important role in the upward NHL trends.

Hepatitis C virus. The association of HCV with some B-cell NHL has been demonstrated by multiple case reports ***(Silvestri et al., 2000; Mizorogi et al., 2000)***. A recent therapeutic study that showed regression of splenic lymphoma with villous lymphocytes after treatment of HCV infection further supports that HCV infection may play a role in lymphomagenesis ***(Hermine et al., 2002)***. However, the findings from epidemiologic studies of a relationship between HCV and NHL are mixed. A positive association between HCV and B-cell NHL has been found in some case-control studies ***(Hausfater et al., 2000;Montella et al., 2001)*** but not in others ***(Pioltelli et al., 2000)***. A study in Southern Italy showed a higher incidence of HCV infection in aggressive NHL than in low-grade NHL ***(Montella et al., 2001)***, whereas other studies report a higher risk for low-grade NHL ***(Hausfater et al., 2000)***.

3-Familial aggregation

A history of NHL or other hematolymphoid cancer in close relatives has been repeatedly shown to increase the risk of NHL by 2- to 3-fold ***(Linet et al., 1992;Paltiel et al., 2000)***. A stronger association than that estimated for most of the other suspected risk factors. The aggregation is associated with an inherited defect of immune function in some instances, although in many families no such abnormality can be discerned ***(Rabkin et al., 1997).***

Lymphomas may also cluster within families, not because of an inherited genetic susceptibility, but because of shared environmental determinants. Studies ***(Paltiel et al., 2000;Zhu et al., 2001)*** have shown that pesticide-related risks and occupational exposure to benzene were greater among individuals with a positive family history of cancer. Ward and colleagues ***(Ward et al., 1994)*** found that the risks of NHL in people with a low intake of vitamin C and carotene were greater among individuals with a family history of cancer, particularly a history of hematolymphoid cancer.

Chiu and coworkers ***(Chiu et al., 2002)*** also found that alcohol use is associated with an increased risk of NHL among only men with a family history of hematolymphoid cancer. These findings are intriguing and suggest that the risk of developing NHL may be determined by interactions between environmental and genetic factors.

However, familial predisposition alone or in conjunction with other environmental or occupational exposures appears to account for only a small proportion of NHL occurrence (less than 5%) and, thus, cannot explain the increasing incidence of NHL ***(Rabkin et al., 1997;Linet et al., 1992)***.

4-Blood transfusion

Studies that have examined the association between a history of blood transfusion and the risk of NHL have produced contradictory findings. Cohort studies have supported the hypothesis that previous allogeneic blood transfusion may increase the risk of developing NHL, but several case-control studies published subsequently failed to replicate the findings ***(Vamvakas et al., 2000)***. Blood transfusion has also been specifically associated with low-grade NHL ***(Brandt et al., 1996;Cerhan et al., 2001)*** or with extranodal high-grade NHL ***(Brandt et al., 1996).***

This increased risk has been attributed to the immunosuppressive effects of allogeneic blood transfusion, as well as the increased susceptibility of those persons who receive transfusions to infections caused by blood borne organisms ***(Cerhan et al., 1993).*** Increases in the use of blood transfusion since the 1950s have coincided with the increase in the incidence of NHL ***(Vamvakas et al., 2000)***.

5-Agricultural and pesticide exposures

An association between NHL and pesticide exposures has been observed repeatedly, but not consistently. Results from a number of epidemiologic studies suggest that the excess risk of NHL is related to the use of phenoxyacetic acid herbicides, organophosphate insecticides, triazine herbicides, and fertilizers ***(Dich et al., 1997)***. A case control study found a strong positive association between serum concentrations of polychlorinated biphenyls and risk of NHL ***(Rothman et al., 1997)***. However, risk estimates vary widely among studies, and in some studies the risk was not increased at all.

A recent study evaluated the pesticide- NHL association according to t(14;18) status and found that t(14;18)-positive NHL was associated with farming and exposures to dieldrin, lindane, atrazine, and fungicides, in marked contrast to null or inverse associations for the same exposures and t(14;18)-negative NHL ***(Schroeder et al., 2001)***.

Even though general population exposures may be lower than those in occupational settings, a relative risk as small as could explain 15% of current NHL risk, assuming that over 90% of the general population is exposed ***(Rabkin et al., 1997).*** Therefore, the role of agricultural and residential pesticides in the etiology of NHL warrants further evaluation.

6-Lifestyle factors:

Little is known concerning the role of diet in the etiology of NHL ***(Davis et al., 1992)***. The risk of NHL has been linked to increased consumption of animal protein, fat, and meat ***(Chiu et al., 1996;Zhang et al., 1999;De Stefani et al., 1998)***. While higher intakes of fruits, cruciferous vegetables, and vegetables high in carotene have shown an inverse association with NHL ***(Ward et al., 1994; Chiu et al., 1996; Zhang et al., 2000)***. Long-term regular use of vitamin supplements appears to have no association with the risk of NHL***(Zhang et al., 2001)*** or mortality from NHL ***(Zhang et al., 2001)***. The data on diet, however, are not conclusive. The most consistent findings to date are that diets high in animal proteins and fats appear to increase the risk of NHL, and fruit and vegetable consumption appears to decrease the risk.

7-Genetic susceptibility

Tumor suppressors and oncogenes. Cytogenetic studies have shown that most NHL exhibit chromosomal abnormalities and that some of the reciprocal chromosomal translocations are closely correlated with specific histological and immunological types ***(Staudt et al., 2002)***. These chromosomal abnormalities include the t(14;18) involving the BCL2 proto-oncogene in 85-90% of follicular lymphomas and 20-30% of diffuse large B-cell lymphomas ***(Meijerink et al., 1997)***; the t(3;14)and other translocations involving the BCL6 protooncogene in 10-15% of follicular lymphomas and 30- 40% of diffuse large B-cell lymphomas ***(Ohno et al., 1997)***; and the t(8;14) and other translocations involving the c-MYC protooncogene in 100% of Burkitt's lymphoma and in 10%- 15% of diffuse large B-cell lymphoma ***(Dalla-Favera et al., 1993)***. It has also been suggested that microsatellite instability contributes to the development of lymphoid malignancies ***(Kodera et al., 1999)***, but the relevance of microsatellite instability in hematological malignancies remains controversial.

Cancer susceptibility genes. Suspected risk factors for NHL include environmental exposures and, therefore, genetically determined variation in the ability to metabolize such exposure may be important in determining NHL risk. These enzymes include the multigene families of cytochrome P450 (CYP) and glutathione S-transferase (GST). CYP1A1 is critical in the activation of polycyclic aromatic hydrocarbons and dioxin ***(Eaton et al., 2000)***, and has been associated with childhood leukemia ***(Infante-Rivard et al., 1999)***. CYP3A4 is involved in the oxidation of parathion and probably other organophosphate insecticides ***(Eaton et al., 2000)***, exposures that have been associated with NHL ***(Zahm et al., 1992)***. CYP2E1 metabolizes a number of carcinogens found in solvents ***(Stubbins et al., 1999)***.

Occupations with exposures to solvents—including chlorinated solvents, benzene, paint thinner, and mineral oil— have been linked to NHL risk ***(Rego et al., 1998)***.

The GST-mediated glutathione conjugation is known to play a role in the detoxification of a broad array of chemicals, including pesticides, polycyclic aromatic hydrocarbons, heterocyclic amines, and solvents ***(Eaton et al., 2000)***. GSTM1 null genotype has been associated with an increased risk of acute myeloid leukemia ***(Arruda et al., 2001)***. Also, GSTT1-null individuals were found to have a more than 4-fold increased risk of a myelodysplastic syndrome, which often progresses to acute myeloid leukemia ***(Chen et al., 1996)***.

8-Other risk factors

Other environmental exposures that have been linked with NHL are generally either rare, only weakly associated, or based on inconsistent reports. Radiation exposure probably has little effect on NHL risk ***(Rabkin et al., 1997)***. Solvents have been associated with an increased risk of NHL, especially in occupational studies of rubber workers, aircraft maintenance workers, and dry cleaners ***(O'Connor et al., 1999)***. The effects of solvent exposure may be through their effect on the immune system. Benzene workers, chemists, farmers, grain handlers, petroleum refinery workers, anesthesiologists, pathologists, and woodworkers are in occupations that have also been linked to an increased risk of NHL ***(O'Connor et al., 1999)***. It was estimated that the portion of NHL that can be attributed to these occupational exposures varies from 4 to 11% ***(Hartge et al., 1992)***.

PATHOLOGIC CLASSIFICATION

Considerable progress has occurred in the classification of non-Hodgkin's lymphomas during the past 15 years. The morphologically oriented **Rappaport system**, which was introduced in the late 1960s, allowed for the separation of non-Hodgkin's lymphomas into two clinically useful categories: those with a favorable prognosis (nodular lymphomas) and those with an unfavorable prognosis (diffuse lymphomas). The National Cancer Institute-sponsored international Working Formulation (WF) classification introduced in 1982 was meant to be a translation system for other classifications, including the Rappaport and the immunologically oriented Lukes and Collins and Kiel systems ***(The NHL Pathologic Classification Project., 1982).***

In 1994, the International Lymphoma Study Group proposed the Revised European-American Lymphoma classification (REAL) schema. The REAL classification was based on known disease entities and promoted the concept that a range of morphologic grades and degrees of clinical aggressiveness might be present within each entity ***(Harris et al., 1994).***

In 1995, the Society for Hematopathology and the European Association of Hematopathologists jointly developed a classification of hematologic neoplasms for the World Health Organization (WHO). The goals of the WHO project, in part, were to update and revise the REAL classification of the lymphoid neoplasms, using a combination of morphology, immunotyping, genetic features, and clinical syndromes. The goal was to define disease entities of B-cells, T-cells, and natural killer (NK) cells that could be recognized by pathologists and that have clinical relevance.To ensure such relevance, a clinical advisory committee of

oncologists with American and European co-chairs reviewed and discussed the proposed WHO classification at a meeting in 1997. Proponents of all major lymphoma and leukemia classifications have agreed that if a reasonable consensus emerged from this effort, they would accept the WHO classification of hematologic malignancies as the standard ***(Harris et al., 1999)***. Of the WHO publications, only major disease categories are listed in one ***(Harris et al., 1999)***; a comprehensive listing of subtypes and variants is presented in a separate publication ***(Jaffe at al.,2001).***

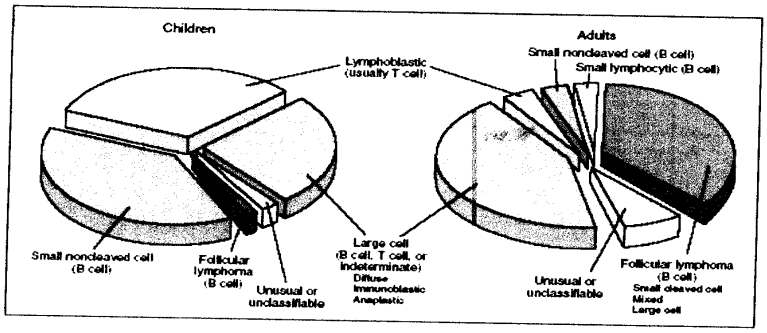


Fig.(2): Distribution of Histologic Subtypes of Non-Hodgkin's Lymphoma in Children and Adults. ***(Magrath et al., 1993)***

Table (1): **Major Histopathological Categories of Non-Hodgkin's Lymphoma in Children and Adolescents**

Category WHO Classification/Updated REAL	Category (Working Formulation)	Immuno-phenotype	Clinical Presentation	Chromosome Translocation	Genes Affected
Lymphoblastic lymphoma, Precursor T/leukemia	Lymphoblastic convoluted and nonconvoluted	T cell	Mediastinal, bone marrow	MTS1/p16ink4a deletion TAL1 t(1;14)(p34; q11), t(11;14)(p13;q11)	TAL1 TCRαo*** RHOMB1, HOX11
		Pre-B-cell	Skin, bone		
Burkitt's and Burkitt's like lymphomas	Malignant Lymphoma small noncleaved cell	Mature B cell	Intra-abdominal (sporadic) jaw (endemic) head and neck (nonjaw) (sporadic)	t(8;14)(q24 q32), t(2;8)(p11;q24), t(8;22)(q24; q11)	c-myc, IgH, IgK, Ig1**
Diffuse large B-cell lymphoma	Malignant Lymphoma large cell	Mature B cell; maybe CD30+	Nodal, abdomen, bone, primary CNS, mediastinal	Not well characterized in children	
Anaplastic large cell lymphoma, systemic	Malignant Lymphoma immuno-blastic or ML large	CD30+ (Ki-1+)	Variable, but systemic symptoms often prominent	t(2;5)(p23; q35)	ALK, NMP
		T cell or null cell			
Anaplastic large cell lymphoma, cutaneous		CD30+ (Ki-usually)	Skin only; single or multiple lesions	Lacks t(2;5)	
		T cell			

(Percy et al., 1999)

Table (2): WHO Classification of Neoplastic Diseases of Hematopoietic and Lymphoid Tissues .

Precursor B-cell neoplasms

Precursor B-lymphoblastic leukaemia/lymphoma

Mature (Peripheral) B-cell neoplasms

1- B-cell chronic lymphocytic leukaemia/prolymphocytic leukaemia/small lymphocytic lymphoma

2- Lymphoplasmacytoid lymphoma

3- Mantle cell lymphoma

4- Follicular lymphoma, (grade 1,2 or 3)

5- Nodal marginal zone B-cell lymphoma: extranodal (MALT-type ± monocytoid B cells); provisional subtype: nodal (± monocytoid B cells)

6- Splenic marginal zone lymphoma (± villous lymphocytes)

7- Hairy cell leukaemia

8- Plasmacytoma / plasma cell myeloma

9- Diffuse large B-cell lymphoma.

Morphologic variants:

- Centroblastic - Immunoblastic - T-cell/histocyte-rich
- Anaplastic large B-cell - Plasmablastic

Clinical variants:

- Mediastinal (thymic) large B-cell lymphoma - primary effusion lymphoma
- Intravascular large B-cell lymphoma - Lymphomatoid granulomatosis type

10- Burkitt's lymphoma Burkitt cell leukemia

Morphologic variants:

- Burkitt like or typical Burkitt - With plasmacytoid differentiation (AIDS-associated)

Clinical and genetic variants:

- Endemic - Sporadic - Immunodeficiency-associated

Precursor T-cell neoplasms

Precursor T-lymphoblastic lymphoma/leukaemia

Mature (peripheral) T-cell neoplasms

1- Predominantly leukemic/disseminated

T-cell prolymphocytic leukemia

T-cell granular lymphocytic leukemia

Aggressive NK-cell leukemia

Adult T-cell lymphoma/leukemia

2- Predominantly nodal

Angioimmunoblastic T-cell lymphoma

Peripheral T-cell lymphoma,not otherwise characterized

Anaplastic large-cell lymphoma, T/null cell, primary systemic disease

3- Predominantly extranodal

Mycosis fungoides/sezary syndrome

Anaplastic large-cell lymphoma, T/null cell, primary cutaneous type

Subcutaneous panniculitis-like T-cell lymphpma

Extranodal NK/T-cell lymphoma, nasal type

Entropathy-type T-cell lymphoma

Hepatosplenic gamma-delta T-cell lymphoma

(Deibold, 2001)

CLINICAL FEATURES

HISTORY AND PHYSICAL EXAMINATION

The history :

Lymphadenopathy — More than two-thirds of patients with NHL present with peripheral lymphadenopathy.

- Rapid and progressive enlargement of lymph nodes portends a diagnosis of an aggressive or highly aggressive NHL.
- Waxing and waning of lymph nodes, including their complete disappearance and reappearance, is commonly seen in the indolent lymphomas ***(Frumkin, 2003).***

Systemic complaints (B symptoms) — About 40% of patients with NHL present with systemic complaints of fever, weight loss, or night sweats (ie, "B" symptoms)***(Frumkin, 2003)***. These complaints are of extreme importance in determining prognosis, and have been formally defined as follows:

- Fever temperature >38
- Weight loss — unexplained loss of >10 percent of body weight over the past 6 months
- Sweats — the presence of drenching night sweats.

Such systemic ("B") symptoms are more common in patients with aggressive and highly aggressive histologies (47 %), especially in those with hepatic and extranodal involvement. In contrast, only about (25 %) of patients with indolent lymphomas have B symptoms. When present, systemic symptoms in patients with indolent lymphoma are usually

associated with extensive advanced stage disease and with bulky masses more than 5-10 cm in greatest diameter.

Less frequent presenting systemic complaints of fatigue, malaise, and pruritus occur in fewer than 10% of patients. Their presence, although important to document, is not as critical for prognosis as are fever, sweats, or weight loss. The presence of bone pain or gastrointestinal symptoms may indicate extranodal involvement in these areas ***(Nakatsuka et al., 2002)***.

Specific illnesses, in addition to the above, which are risk factors for the development of gastrointestinal lymphoma include:

- Crohn's disease
- Gastrointestinal nodular lymphoid hyperplasia
- Helicobacter pylori-associated chronic gastritis
- Celiac disease.

As an example, clinical deterioration of a patient with celiac disease, despite compliance with a gluten-free diet, should raise suspicion of the possible presence of lymphoma.

Pyothorax-associated lymphoma, reported mostly from Japan, is a rare complication of long-standing pyothorax, most often in association with tuberculosis and EBV infection ***(Nakatsuka et al., 2002)***.

Physical examination — needs to be directed to all potentially involved lymphoid sites ***(Urquhart et al., 2001)***, including :

- Waldeyer's ring (tonsils, base of the tongue, nasopharynx).
- Standard lymph node sites (cervical, supraclavicular, axillary, inguinal, femoral).

- Liver and spleen.
- Abdominal nodal sites (mesenteric, retroperitoneal).
- Less commonly involved nodal sites (eg, occipital, preauricular, epitrochlear).

Involvement of head and neck — Nodal and extranodal involvement of the head and neck, including Waldeyer's ring (tonsils, base of the tongue, nasopharynx) is more frequently observed in patients with NHL than in those with Hodgkin's disease (HD) ***(Urquhart et al., 2001)***. While this involvement is often detected only via indirect laryngoscopy, a useful clue to the presence of involvement of Waldeyer's ring is enlargement of preauricular nodes.

Primary central nervous system lymphoma constitutes only 1% of all NHLs. However, with increasing incidence of HIV-1 infection, as well as increasing use of high dose immunosuppressive therapy, primary CNS lymphoma has become one of the most common types of primary brain tumors. Symptoms of primary CNS lymphoma include headache, lethargy, focal neurologic symptoms, seizures, or paralysis. Direct lymphomatous involvement of peripheral nerves (neurolymphomatosis) is a rare event, often occurring in the presence of widespread systemic disease ***(Ghobrial et al., 2004)***.They commonly involves the eye, while lymphoma involving the orbital structures (eg, eyelid, extraocular muscles, lacrimal apparatus, conjunctivae) is rare ***(Bhatia et al., 2002)***. Other uncommon CNS manifestations of NHL as the initial presentation include spinal cord compression occurring in 0.1-6.5% of patients, and lymphomatous meningitis.

Patients with highly aggressive NHL, HIV+ NHL, or aggressive lymphoma who have bone marrow or paranasal sinus involvement, or at least two extranodal disease sites are at risk of having CNS involvement ***(Hegde et al., 2005)***.

Involvement of chest and lungs — Although much less common than with HD, approximately 20% of patients with NHL present with mediastinal adenopathy. These patients can present with persistent cough, chest discomfort, or without clinical symptomatology but with an abnormal chest x-ray. In 3-8% of patients with NHL, a superior vena caval syndrome is part of clinical presentation.

Other associated findings in the thorax include pleural and, less commonly, pericardial effusions. Pleural disease is seen in about 10% of all patients with NHL at diagnosis ***(Civardi et al., 2002)***. The differential diagnosis of mediastinal presentation includes infections (eg, histoplasmosis, tuberculosis, infectious mononucleosis), sarcoidosis, HD, as well as other neoplasms.

Abdominal and pelvic involvement — Involvement of retroperitoneal, mesenteric, and pelvic nodes is common in most histologic subtypes of NHL. Unless massive or leading to obstruction, nodal enlargement in these sites usually does not produce symptoms. However, patients who come to medical attention because of an abdominal mass, massive splenomegaly, or primary gastrointestinal tract lymphoma present with complaints similar to those caused by other abdominal space occupying lesions. These complaints include anorexia, weight loss, nausea and vomiting, chronic pain, abdominal fullness, early satiety, symptoms associated with visceral obstruction, or even acute perforation and gastrointestinal hemorrhage. Occasional patients will present with malabsorption syndrome ***(Civardi et al., 2002)***.

While diffuse hepatosplenomegaly is common in the indolent lymphomas, synthetic liver function is usually intact. In contrast, discrete hepatic masses are more commonly seen in the aggressive or highly aggressive histologies ***(Civardi et al., 2002)***.

However, in one study of 414 consecutive patients with NHL, only 39% of the detected focal liver lesions at disease onset were due to NHL; 58% were benign ***(Civardi et al., 2002)***. Conversely, 74% of the liver lesions detected during follow-up were due to NHL, while 15% were due to a malignancy other than NHL (eg, hepatocellular carcinoma, metastatic lesions from a second malignancy).

Extranodal sites: 10-35% of patients with NHL will have primary extranodal lymphoma at initial diagnosis, and about 50% will have extranodal disease during the course of the disease ***(Seymour et al., 2001)***. The most common site of primary extranodal disease is the gastrointestinal (GI) tract, followed by skin. Symptoms due to extralymphatic disease are usually associated with aggressive NHL, and are uncommon in indolent lymphomas. Other sites involved with aggressive non-Hodgkin lymphomas at presentation include the testis, bone, and kidney.

• Testicular NHL, presenting as a mass, constitutes 1% of all NHLs and 2% of all extranodal lymphomas; it is the most common malignancy involving the testis in men over age 60 ***(Seymour et al., 2001)***.

• NHL of bone is usually the manifestation of disseminated disease, but can present as a solitary lesion, usually with pain, mass, swelling, or pathologic fracture in 20% of cases.

• Clinical evidence of renal involvement occurs in 2-14% of patients with NHL, usually of aggressive or highly aggressive subtypes, but rarely

presents with symptoms of renal failure at diagnosis. Ureteral obstruction due to retroperitoneal disease, or urate nephropathy are more likely causes of renal insufficiency at the time of diagnosis.

• Rare extranodal sites at presentation of non-Hodgkin's lymphoma include the prostate, bladder, ovary, orbit, heart, breast, salivary glands, thyroid, and adrenal glands ***(Bhatia et al., 2002; Wong et al., 2002)***.

• The skin should be carefully examined for lesions; all suspicious areas should be biopsied.

• Up to 35-65% of poorly-differentiated neoplasms of unknown primary site may represent cases of NHL.

OTHER CLINICAL PRESENTATIONS — NHLs may present in a number of nonspecific ways, and may present to the clinician because of abnormal laboratory results, via certain oncologic emergencies, or because of lymphoma-associated paraneoplastic phenomena.

Abnormal laboratory results — Less common presentations of systemic NHL include unexplained anemia, thrombocytopenia or, less commonly, leukopenia due to extensive bone marrow infiltration or hypersplenism from splenic involvement.

Although 15% of patients with NHL develop hypercalcemia, symptoms of hypercalcemia are uncommon at diagnosis, except in HTLV-1 associated adult T-cell lymphoma/leukemia. These symptoms include dehydration, lethargy, weakness, nausea, vomiting and constipation.

Hyperuricemia causing symptoms of gout or nephrolithiasis are unusual at presentation, but more typically can present problems during initial treatment of rapidly proliferating NHLs ***(Ghobrial et al., 2004).***

Potential complications : Complications of NHL need to be considered during the initial workup and evaluation of the patient, particularly those with aggressive and especially highly aggressive histologies ***(Ghobrial et al., 2004)***. Prompt recognition and therapy is critical for these situations, which may be life-threatening, interfere with and/or delay treatment of the underlying NHL. These can include:

- Spinal cord compression
- Pericardial tamponade
- Hypercalcemia (adult T-cell lymphoma/leukemia)
- Superior or inferior vena cava obstruction
- Hyperleukocytosis (B- or T-cell lymphoblastic lymphoma)
- Acute airway obstruction (mediastinal lymphoma)
- Lymphomatous meningitis and/or CNS mass lesions
- Hyperuricemia and tumor lysis syndrome
- Hyperviscosity syndrome (lymphoplasmacytic lymphoma with Waldenstrom's syndrome)
- Intestinal obstruction, intussusception
- Ureteral obstruction, unilateral or bilateral hydronephrosis
- Severe hepatic dysfunction
- Severe autoimmune hemolytic anemia and/or thrombocytopenic purpura (B-cell small lymphocytic lymphoma) ***(Ghobrial et al., 2004)***.

Paraneoplastic syndromes — An uncommon presentation of NHL would be manifestation of a paraneoplastic syndrome. The syndromes which are seen in patients with NHL include neurologic, hematologic, renal, dermatologic, gastrointestinal, and rheumatologic manifestations. ***(Hegde et al., 2005)***.

DIAGNOSIS

LYMPH NODE AND TISSUE BIOPSY

Lymph node selection : dependent upon the clinical situation, characteristics of the patient (age, gender), as well as the location of the involved node(s). Regardless of whether lymphadenopathy is localized, regional, or more generalized, if other diagnostic tests do not support another diagnosis*(Jaffe et al., 2001)*, a lymph node should be considered for biopsy if one or more of the following is present:

- The node is significantly enlarged
- The node persists for longer than four to six weeks
- The node is progressively increasing in size.

Studies on excised tissue — Accurate histopathologic evaluation of sufficient neoplastic tissue, preferably an intact lymph node, is critical. Although a tissue diagnosis of "lymphoma" can often be made by fine needle aspiration (FNA) or core needle biopsy, only an excisional biopsy of an intact node consistently allows sufficient tissue for histologic, immunologic, molecular biologic assessment, and classification by experienced hematopathologists *(Jaffe et al., 2001)*. The importance of such a complete evaluation for appropriate diagnostic, prognostic, and treatment purposes cannot be overemphasized *(Siebert et al., 2001; Lester et al., 2003;Hehn et al., 2004)*.

Types of Biopsy Used to Diagnose Non-Hodgkin Lymphoma

- Fine needle aspiration biopsy.
- Excisional or incisional biopsy.
- Bone marrow aspiration and biopsy.
- Lumbar puncture (spinal tap).
- Pleural or peritoneal fluid examination. ***(Weinstein et al., 2001).***

INITIAL LABORATORY STUDIES — After the initial biopsy, the following baseline blood tests should be obtained:

- Complete blood count, differential WBC count , and examination of the peripheral smear for the presence of atypical cells, suggesting peripheral blood and bone marrow involvement
- Biochemical tests of renal and liver function, including Blood Urea Nitrogen (BUN), creatinine, alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and albumin
- Serum calcium, electrolytes, and uric acid. ***(Cairo et al., 2003).***

These tests may not only contribute to staging but may also influence the type of therapy chosen and its timing (eg, hyperuricemia), and guide further investigations to other potential sites of disease.

Surrogate tumor markers — Two serum tests have had specific value in the various lymphomas, serum beta-2 microglobulin and lactate dehydrogenase ***(Dumontet et al., 1999)***.

1. The serum beta-2 microglobulin level is useful in patients with indolent lymphomas, where it has been used for prognostic purposes, as a surrogate measure of disease volume, and for monitoring response to therapy.
2. Serum concentrations of lactate dehydrogenase (LDH) have been identified as an important independent predictor of survival in NHL. Increases in LDH isoenzymes 2 and 3 appear to be important pretreatment prognostic variables for survival in NHL, whereas increases in isoenzymes 4 and 5 reflect myeloid regeneration following chemotherapy ***(Dumontet et al., 1999)***.

Immunoglobulin studies — In patients with small lymphocytic lymphoma, a serum protein electrophoresis is useful to document a circulating monoclonal paraprotein or hypogammaglobulinemia, especially since the latter may place the patient at increased risk for infection. Patients with lymphoplasmacytoid lymphoma/Waldenstrom's macroglobulinemia may have an IgM paraprotein in the serum which may be sufficiently high to be responsible for hyperviscosity symptoms ***(Falini et al., 2002)***.

Immunologic and molecular studies — Biologic studies, including cell surface markers, cytogenetics, and molecular techniques are currently integrated into the classification, diagnosis, staging, and detection of minimal disease in the NHLs ***(Harris et al., 1999)***. Monoclonal antibodies directed against cell surface antigens expressed on lymphoid cells, and molecular techniques to define immunoglobulin and T-cell receptor gene rearrangements are also sensitive tools with which to assess tumor cell

infiltration more accurately than histologic examination alone *(Falini et al., 2002)*.

Flow cytometry: Flow cytometry may be used to examine the cells of a biopsy sample. It can help determine whether the lymph node is enlarged because of non-Hodgkin lymphoma, a benign growth (a noncancerous disease), or some other cancer. It also can help to determine the exact type of non-Hodgkin lymphoma so that they can select the proper treatment. The cells being examined are treated with special laboratory antibodies and are passed in front of a laser beam. Each antibody sticks only to certain lymphoid cells. If the sample contains those cells, the laser light will cause them to give off light of a different color, which is measured exactly and analyzed by a computer *(Cairo et al., 2003)*.

Immunohistochemistry (IHC): As in flow cytometry, a part of the biopsy sample is treated with special laboratory antibodies. But instead of using a laser and computer for analysis, the cells are treated so that certain types of cells change color. The color change is seen under a microscope. Like flow cytometry, IHC is helpful in distinguishing different types of non-Hodgkin lymphoma from one another and from other diseases *(Cairo et al., 2003)*.

Cytogenetics: This test helps in identifying certain types of non-Hodgkin lymphoma by looking at cells' chromosomes (pieces of DNA and protein that control the cells' growth and metabolism). In certain types of lymphoma, part of one chromosome may be attached to part of a different chromosome (DNA translocation). This can be seen under a microscope or, as is more common now, detected by chemical methods *(Cairo et al., 2003)*.

Molecular genetic studies: Tests of lymphoma cell DNA can also detect translocations. DNA tests such as polymerase chain reaction (PCR) or fluorescent in situ hybridization (FISH) can find some translocations involving parts of chromosomes too small to be seen with usual cytogenetic testing under a microscope. This sophisticated test is not needed to diagnose most childhood lymphomas, but it is sometimes helpful in lymphoma classification because some subtypes of lymphoma have distinctive translocations ***(Cairo et al., 2003).***

IMAGING STUDIES

- Chest x-ray.

- Ultrasound (ultrasonography).

- Computed tomography (CT or CAT) scan and spiral CT.

- Magnetic resonance imaging (MRI) scan. ***(Cairo et al., 2003).***

- **Positron emission tomography**: PET scans use glucose that contains a slightly radioactive atom. The glucose solution is injected into a vein and travels throughout the body. Cancer cells absorb high amounts of the radioactive sugar because of their high rate of metabolism. A special camera can then detect the radioactivity.

A PET scan can be more helpful than several different x-rays because it scans for lymphoma throughout the whole body. It can also tell if an enlarged lymph node contains lymphoma or is benign. Some centers are now combining it with CT scans to get a better quality image. PET is also used after treatment in helping decide whether an enlarged lymph node still contains lymphoma or is merely scar tissue. Although this test is

relatively new, it is becoming widely used to examine people with lymphomas *(Zinzani et al., 2004)*.

Numerous studies indicate that positron emission tomography (PET) using 18F-fluorodeoxyglucose (18F-FDG) detects actively metabolizing tumor in residual masses following or during *(Haioun et al., 2003)* chemotherapy, and that persistent abnormal uptake predicts for early relapse and/or reduced survival *(Zinzani et al., 2004)*. PET appears to be sensitive for detecting NHL in extranodal sites, although its reliability for detection of bone marrow involvement has been questioned *(Carr et al.,1998; Elstrom et al., 2003)*. In three studies, PET outperformed Gallium scanning *(Kostakoglu et al., 2002;Wirth et al., 2002;Zijlstra et al., 2003)* and rivaled CT *(Kostakoglu et al., 2002)* in its capability to detect disease sites in intermediate to high-grade NHL and Hodgkin's disease, although the additional findings did not affect patient management.

Other imaging techniques : Other tests are becoming more important in both staging and evaluation of patients with NHL *(Bangerter et al., 1999)*:

- Radionuclide scanning, especially with gallium, appears to have clinical utility. Gallium scans are positive in virtually all aggressive lymphomas and in about 50% of indolent lymphomas *(Hsu et al., 2002)*. Gallium scans combined with single photon emission computed tomography (SPECT) are very sensitive in detecting tumor infiltration. These tests are also useful in monitoring response to therapy *(Israel et al., 2002)* as well as for evaluation of residual masses following completion of therapy *(Zinzani et al., 1999)*.

Staging of Non-Hodgkin Lymphoma in Children

Staging is the process of gathering all the diagnostic information to determine how far the lymphoma has spread. This helps the medical team determine the best treatment and the outlook for cure. Many cancers are staged with a system called the TNM system developed by the American Joint Committee on Cancer. But lymphomas are staged by other systems, developed by specialists in lymphoma diagnosis and treatment ***(Murphy et al., 1978)***. The staging system often used to describe the spread of non-Hodgkin lymphoma in children is called the ***St.Jude staging system:***

Stage I: Non-Hodgkin lymphoma starting in one place, either as a single tumor not in lymph nodes or in lymph nodes in one part of the body (the cervical, inguinal, axillary, etc.). The lymphoma is not in the chest or abdomen.

Stage II: In Stage II, the lymphoma is not in the chest. There is a single tumor mass not in lymph nodes that has spread to one nearby group of lymph nodes.

Or The lymphoma started in the intestinal tract and all visible tumor masses have been removed.

Or The lymphoma is growing as 2 separate tumors not in lymph nodes or in more than one group of lymph node where they are on the same side of (above or below) the diaphragm. For example, this might mean cervical and axillary nodes are affected but not the combination of axillary and inguinal nodes.

Stage III: This includes any non-Hodgkin lymphoma that starts in the chest (usually in the thymus or lymph nodes in the center of the chest or the lining of the lung)

Or Lymphoma starting in the abdomen that has spread widely within the abdomen and cannot be completely removed by surgery

Or Lymphoma located next to the spine

Or The lymphoma is in 2 groups of lymph nodes on different sides of the diaphragm

Or Two lymphoma tumors, not in lymph nodes, that are on different sides of the diaphragm

Stage IV: This includes any non-Hodgkin lymphoma that has spread to the bone marrow (but less than 25% of the cells are lymphoma) or the central nervous system (brain or spinal cord). ***(Cairo et al., 2003).***

Grading of tumor burden — A method for stratifying patients with low or high tumor burden is that of the Groupe d'Etude Lymphomes Folliculaire (GELF) ***(Brice et al., 1997)***. In this system, any one of the following characteristics qualifies as a high tumor burden:

- Systemic symptoms
- three or more lymph nodes sites >3 cm in diameter
- A single lymph node site >7 cm in diameter
- Platelets <100,000/μL or absolute neutrophil count <1,000/μL
- Circulating lymphoma cells >5,000/μL
- Marked splenomegaly, compressive symptoms, pleural effusion, or ascites.

Treatment of Non-Hodgkin Lymphoma in Children

Non-Hodgkin lymphoma cells are probably present in other organs, but these are too small to be felt by the doctor or seen on imaging tests. That is why treatment is given to the entire body. This type of treatment is called systemic drug therapy (chemotherapy). It is the only way to kill all of these cells ***(Bethesda, 2005).***

Surgery

Surgery is usually not done to treat non-Hodgkin lymphoma, as it is unlikely to be curative and normal organs might be damaged in the process ***(American Cancer Society, 2005)***. The major reasons for surgery include the following:

- To obtain biopsy tissue for diagnostic tests to determine the exact type of non-Hodgkin lymphoma when nonsurgical procedures (FNA, bone marrow biopsy, etc.) could not obtain enough tissue.

- On an emergency basis, to relieve a blockage (obstruction) in the child's intestine caused by a mass of non-Hodgkin lymphoma.

Radiation Therapy

Radiation focused on a cancer from a source outside the body is called external beam radiation. This is the type of radiation therapy most often used to treat non-Hodgkin lymphoma. Radiation was once used for treating children with non-Hodgkin lymphoma, but no longer. Sometimes, it is used in low doses, along with chemotherapy.

Radiation therapy can also be used to ease (palliate) symptoms caused by lymphoma involving internal organs, such as the brain or spinal

cord, or when it is causing pain because it is pressing on nerves. Immediate side effects of radiation therapy may include mild skin problems or fatigue. Radiation of the abdomen may cause upset stomach and diarrhea. Often these effects go away after a short while ***(American Cancer Society, 2005)***.

Chemotherapy

The treatment for children with non-Hodgkin lymphoma uses a combination of several chemotherapy drugs given over a period of time. Chemotherapy drugs kill cancer cells but also damage normal cells and cause side effects. These side effects depend on the type and dose of drugs and how often and how long they are given. Drugs used in cancer chemotherapy attack cells that are dividing to produce new cells. These drugs are useful because lymphoma cells divide and reproduce more often than do normal cells ***(American Cancer Society, 2005)***.

However, some normal cells, such as those in the bone marrow, the lining of the mouth and intestines, the skin, and the hair follicles, also divide often. These dividing cells are the ones most readily damaged by chemotherapy. As a result, a patient may have:

- hair loss (alopecia). - mouth sores. - diarrhea. – nausea.

- lowered resistance to infection due to low white blood cell counts.

- easy bruising and bleeding due to low platelet counts.

- fatigue due to low red blood cell counts.

- **Growth factors** can be given to keep the blood cell counts higher.

Tumor lysis syndrome results from rapid breakdown of malignant cells resulting in a number of metabolic abnormalities, most notably hyperuricemia, hyperkalemia, and hyperphosphatemia. Hyperhydration and allopurinol or rasburicase (urate oxidase) are essential components of therapy in all but patients with the most limited disease ***(Pui et al., 2001;Goldman et al., 2001).*** A strategy used in Europe is to treat patients with an initial prephase consisting of low-dose cyclophosphamide and vincristine, but this does not obviate the need for allopurinol or rasburicase and hydration. Gastrointestinal bleeding, obstruction, and (rarely) perforation may occur. Hyperuricemia and tumor lysis syndrome, particularly when associated with ureteral obstruction, frequently result in life-threatening complications. Patients with NHL should be managed only in institutions having pediatric tertiary care facilities.

There are possible long-term effects of treatment to organs such as the kidneys, liver, testicles, ovaries, brain, heart, and lungs. With careful monitoring, life-threatening side effects are rare. If serious side effects occur, the chemotherapy may have to be reduced or stopped, at least temporarily. Careful monitoring and adjustment of drug doses are important because some side effects to organs are permanent. Some effects, however, such as testicular damage, may not be able to be avoided ***(American Cancer Society, 2005)***.

One of the most serious side effects is the possibility of developing a second cancer known as acute myeloid leukemia (AML).

All 3 types of childhood non-Hodgkin lymphoma are treated with systemic combination chemotherapy; the main difference is in which drugs are used and for how long they are given ***(Bethesda, 2005).***

Treatment of Stages I and II Lymphoblastic Lymphoma:

All children with stages I and II lymphoblastic lymphoma are assumed to have more widespread disease. They are treated with systemic chemotherapy using combinations of drugs. Typical combinations include "CHOP" (cyclophosphamide, doxorubicin, vincristine and prednisone) or "COMP" (cyclophosphamide, vincristine, methotrexate, and prednisone). But, combinations containing even more drugs might be used. The length of treatment is prolonged and may last as long as 2 years. Around 85% to 90% of children at this stage will be cured. Chemotherapy is also given into the spinal fluid ***(Link et al., 1997).***

Treatment of Stages III and IV Lymphoblastic Lymphoma:

The treatment for children with advanced lymphoblastic lymphoma is always lengthy, lasting up to 2 years. Treatment is given as 3 phases of chemotherapy with multiple drugs. The BFM regimen is often used, but there is another phase of intense treatment after the first few months. Chemotherapy is also given into the spinal fluid. The cure rate is around 60% to 80% ***(Grenzebach et al., 2001).***

Treatment of Stages I and II small Non-cleaved Cell Lymphoma:

Treatment of more advanced stage lymphomas, although very effective, often involves surgery and chemotherapy. If there is a large abdominal tumor, it is important that as much as possible be removed. After that, treatment with chemotherapy is very successful with a cure rate of over 90%. The usual length of treatment is short, ranging from 9 weeks to 6 months. Usually several drugs are used. Most pediatric oncologists feel that the 9-week treatment is adequate. Chemotherapy into the spinal fluid is

needed only if the lymphoma is growing around the head or neck ***(Woessmann et al., 2005).***

Treatment of Stages III and IV small Non-cleaved Cell Lymphoma:

The cure rate for children with this type and stage of non-Hodgkin lymphoma is 70% to 80%. The lymphoma cells in small non-cleaved lymphoma have a very rapid growth rate; therefore, children with this type of non-Hodgkin lymphoma need intense therapy, with little rest between courses of treatment. One successful treatment plan, known as the St. Jude "Total B," alternates cyclophosphamide, doxorubicin, and vincristine with cytarabine (ara-C) and methotrexate every 3 to 4 weeks for a total of 6 to 8 months. Another treatment regimen is the BFM protocol. Chemotherapy must also be given into the spinal fluid ***(Spreafico et al., 2002).***

Treatment of Stages I and II Childhood Large Cell (including anaplastic) Lymphoma:

The cure rate for children with this lymphoma is over 90%. Treatment consists of a short course of chemotherapy with only 4 (sometimes more are used) drugs given for around 3 to 6 months. The usual drug program contains cyclophosphamide, vincristine, prednisone and doxorubicin or methotrexate. Chemotherapy is given into the spinal fluid only if the lymphoma is near the head or neck ***(Cairo et al., 2003;Woessmann et al., 2005).***

Treatment of Stages III and IV Childhood Large Cell (including anaplastic) Lymphoma:

The cure rate for children with advanced large cell lymphoma is 70% to 80%. Large cell lymphoma may involve the bone marrow or spinal fluid. Some oncologists treat large cell lymphoma as they would small

noncleaved lymphoma (if it is B-cell large cell lymphoma) or lymphoblastic lymphoma (if it is T-cell large cell lymphoma).

Intrathecal chemotherapy is important, and systemic drug treatment usually involves doxorubicin, prednisone, vincristine, methotrexate, and possibly 6-mercaptopurine or cyclophosphamide over 9 to 12 months. Current clinical trials are focusing on the length of treatment, which drugs are important in treating large cell lymphoma, and whether T- and B-cell types can be treated similarly ***(Coiffier et al., 2002).***

Bone Marrow Transplantation and Peripheral Blood Stem Cell Transplantation:

These treatments are used for patients who relapse during or after treatment for non-Hodgkin lymphoma. They allow doctors to use higher doses of chemotherapy than would normally be tolerated. High-dose chemotherapy destroys the bone marrow, which prevents new blood cells from being formed. Doctors then repopulate the bone marrow by giving the patient an infusion of blood-forming stem cells after treatment. These are able to create new bone marrow cells. These stem cells can either be autologous stem cell transplant or allogeneic stem cell transplant ***(Bethesda, 2005).***

Autologous stem cell transplant: In an **autologous bone marrow transplant (BMT)** or **peripheral blood stem cell (PBSC) transplant**, blood-forming stem cells are removed from the patient's bone marrow or bloodstream before treatment. The bone marrow stem cells or PBSCs are carefully frozen and stored.

The patient then receives high doses of chemotherapy and sometimes radiation treatment. This destroys all cells in the bone marrow in addition to any remaining cancer cells. The frozen cells are thawed and returned to the patient as a blood transfusion after the high-dose chemotherapy and possible radiation therapy. This type of transplant is more common than an allogeneic transplant ***(Bethesda, 2005).***

Allogeneic stem cell transplant: A transplant using cells from another person is called an **allogeneic BMT** or **allogeneic PBSCT**. Cells from another person may be used when lymphoma cells are found in a patient's own bone marrow in order to avoid returning cancer cells to the child after treatment.

If the patient has a brother or sister who has an identical tissue type, the sibling's bone marrow cells (or possibly PBSCs) can be used instead of the patient's own cells. If a parent is a close match to the patient, the parent's cells can be used. Sometimes cells from a matched but unrelated donor may also be used. Bone marrow or peripheral blood stem cell transplant is a complex treatment, requiring great skill ***(Bethesda, 2005).***

Treatment of Recurrent Lymphoma

Generally, if the lymphoma relapces after curative therapy, it is much harder to treat. A clinical trial is the most appropriate approach. Often this will include some kind of intensive therapy followed by blood-forming stem cell transplantation ***(Levine et al., 2003).***

Complementary and Alternative Therapies

Complementary and alternative therapies are a diverse group of health care practices, systems, and products that are not part of usual medical treatment. They may include products such as vitamins, herbs, or

dietary supplements, or procedures such as acupuncture, massage, and a host of other types of treatment. There is a great deal of interest today in complementary and alternative treatments for cancer. Many are now being studied to find out if they are truly helpful to people with cancer. Most of them are of unproven benefit ***(Bethesda, 2005).***

The American Cancer Society defines **complementary** medicine or methods as those that are used along with regular medical care. If these treatments are carefully managed, they may add to comfort and well-being.

Alternative medicines are defined as those that are used instead of regular medical care. Some of them have been proven not to be useful or even to be harmful, but are still promoted as "cures." If you choose to use these alternatives, they may reduce your child's chance of fighting the cancer by delaying, replacing, or interfering with regular cancer treatment ***(Bethesda, 2005).***

Prognosis of Non-Hodgkin Lymphoma in Children

The determination of prognosis for each of the NHL variants is known to be related to the multiple differences in tumor cell biology (eg, cytogenetics, immunophenotyping, growth fraction, cytokine production) which are found within each of the variants. Therefore, prognostic indicators in the NHLs will take three semi-independent formats:

- A general prognostic score with value in all of the NHL variants, such as the International Prognostic Index.
- A disease-specific prognostic score, with variables reflecting differences in tumor biology for each of the NHL variants ***(Gascoyne et al., 1999)***.
- A treatment-specific prognostic score, with variables reflecting interactions among patient, tumor, and therapeutic regimen ***(Lee et al., 1999)***.

International prognostic index — Institutions in the United States, Canada, and Europe participated in an International Non-Hodgkin's Lymphoma Prognostic Factors Project ***(A predictive model for aggressive non-Hodgkin's lymphoma NEJM, 1993)***. Patients with aggressive NHLs were evaluated for pretreatment features which predicted for survival following treatment with doxorubicin-containing chemotherapy regimens. The following factors were found to correlate significantly with shorter overall or relapse-free survival:

- Age > 60.
- Serum lactate dehydrogenase (LDH) concentration greater than normal.
- ECOG performance status ≥2.
- Ann Arbor clinical stage III or IV.
- Number of involved extranodal disease sites >1.

In this system, one point is given for each of the above characteristics present in the patient, for a total score ranging from zero to five, representing increasing degrees of risk:

- Low risk — IPI score of zero or one
- Low intermediate risk — IPI score of two
- High intermediate risk — IPI score of three
- High risk — IPI score of four or five

IPI for limited stage disease: Modifications of the IPI have been made for patients with limited stage (ie, stage I or II) aggressive NHL ("stage-adjusted" or "stage-modified" IPI) ***(Miller et al., 1998;Moller et al., 2003)***. In one proposed scoring system, one point was given for each of the following pre-treatment variables:

- Age >60
- Increased serum lactate dehydrogenase levels
- Stage II disease
- Performance status ≥2

Prognostic utility of this stage-modified IPI has also been shown for primary gastric and intestinal diffuse large B-cell lymphoma ***(Cortelazzo et al., 1999;Cortelazzo et al., 2002)***, as well as primary intestinal lymphoma of low-grade marginal zone type ***(Cortelazzo et al., 1999)***.

Additional prognostic markers — As newer tests become available and are applied to groups of patients with NHL, one or more may have a prognostic value equal to or greater than those comprising the International prognostic index (IPI).

Serum concentrations of nm23-H1, a protein with nucleoside diphosphate kinase enzyme activity involved in tumor metastasis regulation, are higher than normal in patients with NHL, being lowest in the indolent lymphomas, increasing progressively in the aggressive and highly aggressive variants ***(Niitsu et al., 2001)***. Five years overall survival in patients with diffuse large B-cell NHL (DLBCL) and levels of nm23-H1 ≤80 versus >80 ng/mL was 78 and 6 percent, respectively. In multivariate analysis, a serum concentration ≥12.01 ng/mL was associated with a higher hazard ratio for reduced overall survival (4.6) than that predicted by the IPI (1.9) ***(Niitsu et al., 2001)***.

Two other candidates are the serum concentration of basic fibroblast growth factor (bFGF, FGF-2) and vascular endothelial growth factor (VEGF), potent stimulators of angiogenesis in vivo ***(Salven et al., 1997; Salven et al., 1999)***. In a multivariate analysis of 200 patients with NHL, those with serum levels of both bFGF and VEGF in the highest quartiles had a relative risk of death (RR 2.9) which was higher than the relative risks associated with any of the components of the IPI ***(Salven et al., 2000)***.

Gene expression profiling — Gene expression profiling (GEP) by means of DNA microarrays is a new approach to classification and diagnosis of NHL and other malignancies ***(Thieblemont et al., 2004)***. An example of its potential usefulness in the NHLs is a subclassification of diffuse large B-cell lymphoma (DLBCL) into "germinal center" or "activated" B-cell types, based on the pattern of gene expression detected by GEP ***(Staudt et al., 2003)***. Patients with germinal center B-like DLBCL, as classified by the GEP technique, had significantly better overall survival than those with the activated B-like variant .This approach also effectively indicated those patients with DLBCL within specific IPI risk categories

who were more likely to respond to treatment of their disease with CHOP-based therapy ***(Shipp et al., 2002)***.

GEP may also be useful for investigating the cellular microenvironment of the tumor, rather than the tumor cells themselves. This was shown in a study using GEP in tumor specimens obtained from patients with untreated follicular lymphoma, in which the length of survival correlated with the molecular features of nonmalignant immune cells (eg, T cells, monocytes, dendritic cells) present in the tumor at the time of diagnosis ***(Kuppers et al., 2004;Dave et al., 2004)***.

Association between Hepatitis C Virus and Non Hodgkin Lymphoma

One of the extrahepatic diseases in which HCV has been implicated is B-cell non-Hodgkin's lymphoma (NHL). HCV associated lymphomas have been observed, but whether they are caused by HCV remains to be shown definitively. There is a suggestion that some B-cell NHL associated with HCV arise from clonal expansion of B-cells with particular immunoglobulin gene rearrangements specific for the E2 protein of the HCV envelope ***(Ivanovski et al., 1998;Quinn et al., 2001)***, which is consistent with the hypothesis that lymphomas develop when B cells proliferate in response to antigen. However, no biological mechanism of HCV-associated lymphomagenesis has been definitively elucidated.

The relationship between HCV infection and lymphoproliferative diseases was first reported by ***Ferri et al in 1994*** and was subsequently highlighted by other Italian ***(Vallisa et al., 1999)***, Japanese ***(Izumi et al., 1997)***, American ***(Zuckerman et al., 1997)*** and Turkish ***(Timuraglu et al., 1999)*** authors, with the prevalence of HCV infection ranging from 9% to 42%. Conversely, research carried out in Great-Britain ***(McColl et al., 1999)***, Canada ***(Collier et al., 1999; Shariff et al., 1999)*** and the USA ***(King et al., 1998)*** did not identify a relationship between HCV and lymphoma, suggesting that the prevalence, environmental and ethnic factors, the distribution of the different virus genotypes, mutations and other infectious agents could account for the geographic variation ***(Hanley et al., 1996; McColl et al., 1999)***.

A large number of studies have generated conflicting results on the association between HCV and NHL, a positive association being found in some of them but not in others. This discrepancy seems to be the result of restrictions to several geographical areas. The incidence of malignant transformation varies in certain areas of the world between 7.4% and 37%. In a large study in France the prevalence of HCV infection in patients with B-cell Non- Hodgkin lymphoma was low 1.83% ***(Hausfater et al., 2001)***. HCV was not considered to play an important role in lymphoma genesis. Another study in Italy found a 3.1 times higher incidence of HCV infection in patients with B-cell Non-Hodgkin lymphoma than in controls confirming a positive association ***(Mele et al., 2003)***. However, the same group of researcher found also a positive association between HCV and other lymphoid and myeloid malignancies comparing with control groups ***(Bianco et al., 2004)***. The wide spectrum of HCV related lymphomas include:

1) the low grade lymphomas preceded by long standing symptomatic EMC type II and
2) the "idiopathic" non-cryoglobulinemic intermediate to high grade lymphomas.

The intimate pathogenetical mechanism involved in HCV-lymphomas remains unknown. HCV is not an oncogenic virus but may excert oncogenic potential via two possible mechanisms:

a) an indirect mechanism which does not necessary implicates infection of target cells and
b) a direct one which hypothesizes that lymphoma is the result of the direct infection of the cells.

Potential mechanisms of pathogenesis of Hepatitis C -associated Lymphoma:

HCV is a member of the RNA flavivirus family. The virus lacks reverse transcriptase, and hence is unable to integrate into the host genome and does not encode for any known oncogenes. The pathogenesis of hepatocellular carcinoma associated with HCV has been much studied, but still remains largely unknown. There is increasing evidence that HCV-encoded proteins may contribute to the pathogenesis of hepatocellular carcinoma. HCV proteins can interfere with signal transduction, growth regulation and apoptosis. HCV proteins, for example the core protein, have the ability to transform mouse fibroblast cells in vitro, and transgenic mice expressing HCV core proteins have, in some studies, developed liver tumors ***(Lai et al., 2002)***.

First important mechanism that involves HCV in lymphomagenes is ***ASSOCIATION WITH SPECIFIC CHROMOSOMAL MUTATIONS***: There is evidence that HCV can induce clonal proliferation of B-cells in patients carrying the virus chronically, with molecular alterations in the lymphocytes that may subsequently play a role in the multi-step process of malignant lymphocyte transformation. Lymphocytes in intra-hepatic follicles in livers of patients with chronic HCV undergo an oligoclonal proliferation ***(Murakami et al., 1999)***. Circulating lymphocytes in patients with chronic HCV, but without evidence of frank lymphoma, overexpress the anti-apoptotic protein bcl-2, with a high incidence of t(14;18) translocations involving the bcl-2 gene ***(Zuckerman et al., 2001)***. There is also a high incidence of circulating monoclonal B cells, as evidenced by populations of lymphocytes expressing the same immunoglobulin heavy chain (IgH) rearrangements. bcl-2 and IgH rearrangements can be cleared from the blood by antiviral therapy, concurrent with suppression of the

HCV, possibly eliminating the early monoclonal proliferation and preventing subsequent transformation to lymphoma ***(Zuckerman et al., 2001)***. Actively replicating virus has been demonstrated in HCV-associated lymphomas ***(Karavattathayyil et al., 2000)***, a finding that although important does not necessarily imply a causative role of HCV.

However, a recent review of 854 lymphomas in patients with HCV infection, although confirming the predominance of B-NHL with a higher prevalence among patients with cryoglobulinaemia, cryoglobulinaemia, could not document the expected high frequency of follicular lymphoma which is well known associated with t(14;18). The most common HCV associated lymphomas were immunoplasmacytic lymphomas, extranodal marginal zone cell lymphomas (liver, salivary gland) and Waldenström macroglobulinaemia.

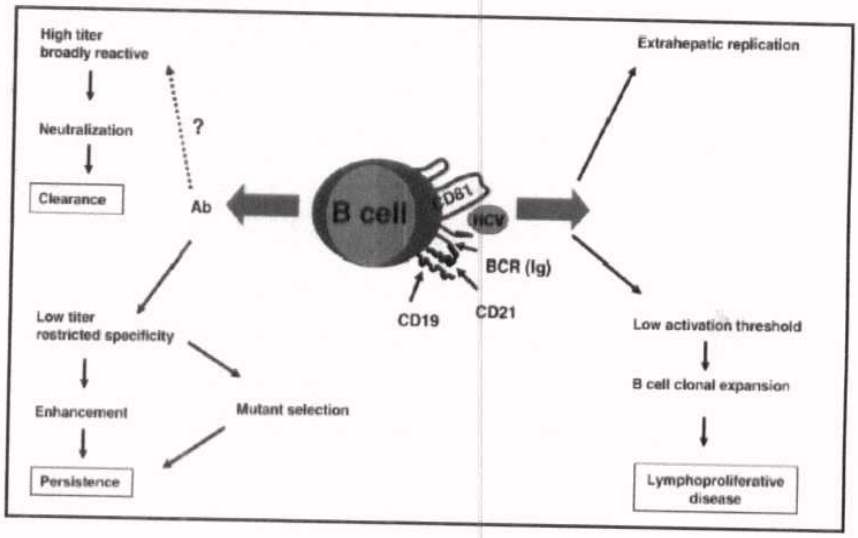
For the second presumed mechanism the key event seems to be the ***CHRONIC ANTIGENIC STIMULATION OF B CELLS BY HCV PROTIENS:*** the most important protein seems to be the HCV-E2 protein ***(Musto et al., 2002)*** which was found to activate B lymphocytes which are considered the origin of HCV associated lymphomas ***(Quinn et al., 2001)***. On the other hand, immunoglobulin variable region genes expressed by B-NHL cells from HCV positive patients, have been shown to exhibit features of ongoing somatic mutations (indicative of antigen selection), as well as the use of a restricted set of variable region genes, indicative of the presence of a common antigen ***(Ivanovski et al., 1998)***. The CD81 protein expressed on the surface of various cells including lymphocytes was found to represent one of the HCV receptors. This protein is implicated in B cell activation ***(Pileri et al., 1998)***.

The third proposed mechanism implicates ***HCV INCLUDED IN THE TARGET CELL WHICH IS SUPPOSED TO UNDERGO TRANSFORMATION***: HCV sequences have been found in lymph node biopsies from patients with B-NHL ***(De Vita et al., 1982; Luppi et al., 1996)*** and HCV-associated proteins were found in lymphoma cells ***(Sansonno et al., 1996)***. In one of the 9 primary liver lymphomas in HCV of infected patients the virus has been detected in the lymphoma cells and not in the surrounding hepatocytes with in situ hybridization assay ***(Ohsawa et al., 1998)***. Moreover, a line of transgenic mice that express the HCV transgene has been found to develop malignant lymphoma with a high frequency within 20 months ***(Ishikawa et al., 2003)***. HCV core mRNA was found in the enlarged lymph nodes of these animals.

However, at present, although the association of HCV with cryoglobulinaemic or non-cryoglobulinaemic lymphoma seems to become clearer, the possible pathophysiological mechanisms are still unknown and mistry.

Regarding therapy, HCV was not found to have significant impact on response to chemotherapy in lymphoma patients. On the other hand, whether to treat low grade lymphomas with anti-viral therapy is debatable. The only well documented study which encourage the role of antiviral treatment in HCV associated lymphoma has been done in patients with splenic lymphoma in whom lymphoma regression occur parallel with the decline of viremia ***(Hermine et al., 2002)***.

Fig (3) : Proposed functions of B cells in HCV infection.



(Mondelli, 2003).

Fig. 3. Production of highly efficient neutralizing antibodies to HCV is currently uncertain although it is likely to occur only in certain circumstances. Such antibodies should be produced at high concentration and possibly have broad specificity, though this is currently unknown. However, in most circumstances neutralizing antibodies would be produced at low concentrations and with restricted specificity; this would on the one hand favour selection of viral mutants and on the other hand it could generate an enhancement mechanism which would increase the spread of the infection to other susceptible cells. In addition to classical antibody production, B cells may undergo aberrant proliferation and rheumatoid factor production, as seen in extrahepatic disease, such as cryoglobulinaemia. To this end, HCV polypeptides such as the E2 glycoprotein and, possibly, NS3 may lower the physiological activation threshold of B cells by interacting with CD81 and the B-cell receptor complex, inducing clonal expansion and, eventually, malignant proliferation as in B-NHL ***(Mondelli, 2003)***.

Table (3): Prevalence of HCV infection in case series of NON-HODGKIN'S LYMPHOMA patients.

TABLE 1- PREVALENCE OF HCV INFECTION IN CASE SERIES OF B-CELL NON-HODGKIN'S LYMPHOMA PATIENTS

Reference	Location	Diagnosis	Number of HCV+/total	%	95% CI
Southern Europe					
Ferri *et al.*[7] (1994)	Pisa, C Italy[1]	B-NHL	17/50	34.0	21.2–48.8
Andriani *et al.*[9] (1996)	Rome, C Italy	B-NHL	10/38	26.3	13.4–43.1
Musolino *et al.*[10] (1996)	Messina S Italy	NHL	5/24	20.8	7.1–42.2
Musto *et al.*[11] (1996)	Puglie, S Italy	B-NHL	40/150	26.7	19.8–34.5
Piolelli *et al.*[12] (1996)	Milano, N Italy	NHL	26/126	20.6	13.9–28.8
Pivetti *et al.*[13] (1996)	Torino, N. Italy	NHL	7/47	14.9	6.2–28.3
De Rosa *et al.*[14] (1997)	Napoli, S Italy	B-NHL	21/91	23.1	14.9–33.1
Mazzaro *et al.*[15] (1997)	NorthEast Italy	NHL	57/199	28.6	22.4–35.4
Silvestri *et al.*[4] (1997)	Udine, N Italy	B-NHL	42/470	8.9	6.5–11.9
Catassi *et al.*[16] (1998)	8 cities, Italy	B-NHL	15/104	14.4	8.3–22.7
De Vita *et al.*[17] (1998)	Aviano N Italy	B-NHL[2]	20/84	23.8	15.2–34.3
Luppi *et al.*[18] (1998)	Modena N Italy	B-NHL	35/157	22.3	16.0–29.6
Pran *et al.*[19] (1999)	Milano, N Italy	Cutaneous B-NHL	1/34	2.9	0.1–15.3
Vallisa *et al.*[20] (1999)	Piacenza, N Italy	B-NHL	65/175	37.1	30.0–44.8
Piolelli, *et al.*[21] (2000)	Lombardy, N Italy	B-NHL	48/300	16.0	12.0–20.6
Montella *et al.*[22] (2001)	Napoli, S Italy	B-NHL	25/101	24.8	16.7–34.3
De Renzo *et al.*[23] (2002)	Napoli, S Italy	B-NHL	12/61	19.3	10.6–31.8
Guida *et al.*[24] (2002)	Bari, S Italy	B-NHL	12/56	21.4	11.6–34.4
Mele *et al.*[25] (2003)	9 cities, Italy	B-NHL	71/401	17.7	14.1–21.8
All Italian studies			529/2,668	19.8	18.3–21.4
Domingo *et al.*[26] (2001)	Spain	B-NHL	12/59	20.3	11.0–32.8
Sanchez Ruiz *et al.*[27] (2001)	Madrid, Spain	B-NHL	9/77	11.7	5.5–21.0
Central Europe					
Tkoub *et al.*[28] (1998)	France	Gastric MALT	1/46	2.2	0.1–11.5
Baudet *et al.*[29] (1999)	Southwestern France	NHL	2/136	1.5	0.2–5.2
Germanidis *et al.*[30] (1999)	France	B-NHL	4/201	2.0	0.5–5.0
Haustaler *et al.*[31] (2001)	Paris, France	B-NHL	3/164	1.8	0.4–5.3
Bronowicki *et al.*[32] (2001)	France	Primary liver B-cell lymphoma	5/24	20.8	7.1–42.2
Ellenrieder *et al.*[33] (1998)	Germany	B-NHL	3/69	4.3	0.9–12.2
Geertsen *et al.*[34] (2001)	Germany	B-NHL	2/105	1.9	0.2–6.7
Zucca *et al.*[35] (2000)	Switzerland	B-NHL	17/180	9.4	5.6–14.7
Paneesha *et al.*[36] (2001)	Manchester	B-NHL	1/112	0.9	0.0–4.9

Northern Europe					
Brind *et al.*[37] (1998)	Newcastle United Kingdom	NHL	0/63[3]	0.0	0.0–5.7
Hanley *et al.*[38] (1999)	Edinburgh, United Kingdom	B-NHL	0/58	0.0	0.0–6.3
McColl *et al.*[39] (1997)	Scotland, United Kingdom	B-NHL	0/69	0.0	0.0–5.3
Singer *et al.*[40] (1997)	Glasgow, United Kingdom	B-NHL	0/31	0.0	0.0–11.2
Thalen *et al.*[41] (1997)	The Netherlands	B-NHL	0/99	0.0	0.0–3.7
Eastern Europe					
Gasztonyi *et al.*[42] (1999)	Hungary	B-NHL	10/42	23.8	12.1–39.5
Cucuianu *et al.*[43] (1999)	Romania	B-NHL	20/68	29.4	19.0–41.7
Asia					
Izumi *et al.*[35] (1996)	Japan	B-NHL	12/54	22.2	12.0–35.6
Izumi *et al.*[44] (1997)	Japan	B-NHL	4/25	16.0	4.5–36.1
Yoshikawa *et al.*[45] (1997)	Japan	B-NHL	9/55	16.4	7.8–28.8
Ogino *et al.*[46] (1999)	Japan	NHL	4/33[4]	12.1	3.4–28.2
Mizoroge *et al.*[6] (2000)	Japan	B-NHL	17/100	17.0	10.2–25.8
Kuniyoshi *et al.*[47] (2001)	Japan	NHL	20/348	5.7	3.5–8.7
Imai *et al.*[48] (2002)	Japan	B-NHL	21/156	13.5	8.5–19.8
All Japanese studies			87/771	11.3	9.1–13.7
Kim *et al.*[49] (2002)	South Korea	NHL	7/214	3.3	1.3–6.6
Udomsakdi-Auewarakul *et al.*[50] (2003)	Thailand	NHL	3/130	2.3	0.5–6.6
Akdogan *et al.*[51] (1998)	Turkey	NHL	4/30	13.3	3.8–30.7
Timuragaoglu *et al.*[52] (1999)	Turkey	NHL	3/35	8.6	1.8–23.1
Arican *et al.*[53] (2000)	Turkey	NHL[5]	2/44	4.5	0.1–15.5
Paydas *et al.*[54] (2000)	Turkey	NHL	9/98	9.2	4.3–16.7
Kaya *et al.*[55] (2002)	Turkey	NHL	1/70	1.4	0.0–7.5
Harakan *et al.*[56] (2003)	Saudi Arabia	B-NHL	12/56	21.4	11.8–34.4
Salem *et al.*[57] (2003)	Lebanon	B-NHL	0/35	0.0	0.0–10.0
Shirin *et al.*[58] (2002)	Israel	B-NHL	10/128	7.8	3.8–13.9
North America					
Collier *et al.*[59] (1999)	Toronto, Canada	B-NHL	0/100	0.0	0.0–3.6
Sharif *et al.*[60] (1999)	British Columbia, Canada	B-NHL	2/88	2.3	0.3–8.0
Zuckerman *et al.*[61] (1997)	Los Angeles, USA	B-NHL	26/120	21.7	14.7–30.1
Kashyap *et al.*[62] (1998)	Los Angeles, USA	B-NHL	36/312	11.5	8.2–15.6
King *et al.*[63] (1998)	Missouri, USA	NHL	1/73	1.4	0.0–7.4
Katamanthavril *et al.*[64] (2000)	New Orleans, USA	B-NHL	8/32	25.0	11.4–43.4
South America					
Chinbum *et al.*[65] (2002)	Brazil	B-NHL	8/87	9.1	4.1–17.3
Studies with NHL and other hemato-poietic neoplasms together					
Markovic *et al.*[66] (1999)	Slovenia	NHL + HD	3/181	1.7	0.3–4.8
Yonac *et al.*[67] (2000)	Turkey	NHL (7%) + HD (1%)	1/92	1.1	0.0–5.9
Rubkin *et al.*[68] (2002)	California, USA	B-NHL (5%) + MM (2%) + HD (1%)	4/95[5]	4.2	1.2–10.4

(Negri et al., 2004)

Table 3 shows the number and percentage of subjects with NHL, in the majority specified as B-cell NHL, that were positive for HCV infection in all case series with more than 20 cases by area and country. Studies were conducted in several countries of Europe, Asia and America, and the number of NHL cases included ranged from 24 to 470. Italy was the country that contributed the largest number of studies. Except for a study restricted to cutaneous B-cell lymphomas, all Italian studies showed a high prevalence of HCV infection among NHL, ranging from 8.9% to 37.1%. In total, 529 out of 2,668 (19.8%) NHL cases were HCV-positive in Italy. In Europe, HCV prevalence was also high in a study from Switzerland (9.4%), 2 studies from Spain (11.7% and 20.3%) and 2 studies from Eastern Europe, 1 from Hungary (23.8%) and 1 from Romania (29.4%). Prevalence of 1–2% was observed in most studies from central European countries, except for a French study of primary liver B-cell lymphoma, where 20.8% of cases were HCV-positive. No HCV-positive patients were observed in over 300 NHL patients from northern Europe, mainly the UK ***(Negri et al., 2004)***.

In Asia, high HCV prevalence was reported in 1 study from Saudi Arabia (21.4%), 1 from Israel (7.8%) and in the 7 studies from Japan (5.7–22.2%). The overall prevalence of HCV infection among NHL patients in Japan was 11.3%. HCV prevalence among NHL patients was lower in a study from South Korea (3.3%), 1 from Thailand (2.3%), and a small Lebanese study (0%), while it ranged between 1.1% and 13.3% in 5 studies from Turkey. Two studies from Canada and one from Missouri, United States, showed low prevalence of HCV infection in NHL patients (0.0 – 2.3%), while 3 studies conducted in New Orleans or Los Angeles found a much higher prevalence (11.5–25.0%). Finally, in a study from Brazil, 9.1% of B-NHL patients were HCV-infected ***(Negri et al., 2004)***.

Management

Management of the lymphoma: In areas of high background HCV prevalence, screening for HCV at diagnosis of all new B-cell malignancies is important to help direct future management, and to predict which patients may develop problems secondary to the HCV during or after treatment ***(Silvestri et al., 1997)***. Patients positive for HCV antibodies should be assessed for HCV viraemia by RT–PCR, although there is no evidence linking baseline viral load and subsequent outcome of treatment. The degree of hepatitis or underlying cirrhosis should be determined, by liver biopsy, in viraemic patients with abnormal liver function tests before therapy is commenced, especially with high-dose chemotherapy ***(Turner et al., 2003).***

The majority of lymphomas presenting concurrently with HCV carriage should be managed in a similar manner to their HCVnegative counterparts. For certain low-grade lymphomas there is increasing evidence that treatment of the HCV with antiviral therapy can lead to remission of the lymphoma. The underlying B-cell monoclonal proliferation associated with EMC can be cleared when the HCV is treated with interferon-α (IFN-α) ***(Mazzaro et al., 1996)***, and there are case reports of long-lasting complete remission of frank lymphoplasmacytoid lymphoma concurrent with eradication of the virus with IFN-α ***(Patriarca et al., 2001)***. Further evidence to support this approach comes from a recent case series of patients with splenic lymphoma with villous lymphocytes, with associated HCV infection and cyroglobulinaemia ***(Hermine et al., 2002)***. In this series, treatment of the HCV with IFN-α and ribavirin was followed by a complete response of the lymphoma in the majority of patients. Patients with the same lymphoma who were not infected with HCV did not respond to the IFN-α therapy. It is not clear if this approach would be applicable to

other lymphomas. There are no data on the relative effectiveness of the treatment, or of the prognosis, or of other subtypes of lymphoma when associated with HCV, and they should be managed in a manner similar to their HCV-negative counterparts ***(Turner et al., 2003)***.

Management of hepatitis C during treatment: The pathogenesis of liver damage secondary to HCV is poorly understood and is a subject of substantial ongoing research. It is possible that hepatitis and liver damage is mediated in part by effects of HCV proteins, and in part by the immune response directed against the virus. The immunosuppression associated with chemotherapy upsets the balance that occurs in every chronically infected patient between viral proliferation and host immune response. During periods of lymphopenia, secondary to chemotherapy or steroids, the virus can proliferate or 'reactivate'. After treatment is completed the immune system reconstitutes, leading to a drop in viral load, and under some circumstances this is accompanied by hepatitis. This contrasts with the hepatitis that can occur at the end of HCV antiviral therapy, which is commonly associated with a rise in viral load. Recovery of the immune system appears to be important in the pathogenesis of liver damage secondary to chemotherapy-induced reactivation of HCV, as biochemical hepatitis usually only becomes apparent after chemotherapy is stopped. This is known as immune reconstitution hepatitis ***(Turner et al., 2003).***

The risk of chemotherapy-induced proliferation of the background virus differs between HBV and HCV. Lymphoma patients undergoing immunosuppressive therapy who are hepatitis B surface-antigen positive will commonly have an immune reconstitution hepatitis. Hepatitis has been reported in 22–78% ***(Lok et al., 1991;Markovic et al., 1999)*** of cases, with a reported mortality rate of 4% ***(Lok et al., 1991)***. Severe reactivation of

HCV on the other hand is uncommon, with a severe flare of hepatitis reported in only one of 33 patients in a recent series ***(Zuckerman et al., 1998)***.

Chemotherapy can generally be administered safely in well selected patients with background HCV infection, provided they are monitored for viral reactivation and hepatitis during therapy. Fatal fulminant hepatitis secondary to HCV has been reported on cessation of chemotherapy ***(Vento et al., 1996)***, an outcome that is unfortunately somewhat unpredictable. At the time of fulminant hepatitis the viral load is low, presumably secondary to the immune response. The HCV viral load at the time of reconstitution hepatitis is therefore an unreliable test to help differentiate potential causes of the hepatitis ***(Turner et al., 2003)***.

Bone marrow transplantation in patients with chronic HCV is associated with a higher incidence of veno-occlusive disease, especially in cirrhotic patients ***(Frickhofen et al., 2004)***. Following transplantation, patients with HCV subsequently have a high long-term risk of developing cirrhosis ***(Strasser et al., 1999)***. No studies have examined whether suppression of the HCV before chemotherapy or bone marrow transplantation improves outcome. It is possible that patients at high risk of viral proliferation and immune reconstitution hepatitis may benefit from suppression of the virus with IFN-α and ribavirin, although this would be at the expense of exacerbated immunosuppression ***(Turner et al., 2003)***.

Subjects and Methods

Cases population:

All Cases with NHL between November 2004 and November 2005 attending an oncology outpatient clinic at the Mansoura University Children Hospital. Fourty six cases in which 32 males and 14 females, with age range 2-16 years. Case patients were eligible to participate if they were under the age of 17, whether newly diagnosed cases or cases taking their chemotherapy or cases who have completed their treatment on follow up visits.

Cases of NHL were diagnosed by:

1- Clinical presentation.
2- Clinical examination.
3- Labaoratory investigations including: Complete Blood Count, liver function tests, serum creatnine, Bone marrow aspiration examination and tissue biopsy (wether L.N or mass), .
4- Radiological investigation including: CXR, U/S, CT scan and MRI according to the site of tumor.

A sheet for each of all studied cases including the following:

-Age at presentation. - Sex. -Residence.
-Mode of presentation. - Histological diagnosis.
-Radiological diagnosis. -Laboratory investigations.
-The final out come of the disease.
-Therapy given.

Selection of controls:

Thirty seven control subjects free from cancer were selected by sex and birth place from among all patients of the general outpatient clinic Mansoura University Children Hospital in Dakahlia, Egypt. They were frequency-matched to the case group by birth place and sex, with no history of previous blood transfusion or surgical operations. The rationale for selecting general patients, was:

(1) They would be representative of the source population of the cases by region, since both clinics draw patients from the same area and
(2) They would be representative of the general population with respect to HCV infection children.

Laboratory assays:

Within 2 hours of collection, the blood was separated and the serum dividedinto aliquots and stored at _80 _C. Samples were later thawed and tested for anti-HCV antibody by HCV enzyme-linked immunoassay (EIA) third generation (Equipar srl Via G.Ferrari, 21/N – 21047 saronno (Va) Italy), according to the manufacturer's instructions. Samples were then tested for HCV RNA by direct nested reverse transcription-polymerase chain reaction (RT-PCR) (QIAGEN S.p.A Via Grosio, 10/10 .20151 Milano Italy). A positive result by the direct RT-PCR method was considered truly positive, and no further investigation was done. A sample that was negative by both direct RT-PCR and EIA was considered negative.

Princple of the third generation ELISA assay (Equipar - Italy):

Microplates are coated with HCV-specific synthetic antigens derived from " core " and "ns" regions encoding for conservative immunodominant antigenic determinants (Core, NS3, NS4 and NS5).The solid phase is the first treated with the diluted sample and HCV ab are captured, if present, by the antigens.After washing out all the other components of the sample, in the second incubation bound HCV Ab are detected by the addition of anti IgG&M antibody, labelled with peroxidase (HRP).The enzyme captured on the solid phase, acting on the substrate/chromogen mixure, generates an optical signal that is proportional to the amount of anti HCV antibodies present in the sample***(Hollinger et al., 1992).***

Princple of the Nested RT-PCR (QIAGEN - Italy):

1) Extraction:

HCV Qualitative PCR Protocol (QlAamp Viral RNA Mini Spin Protocol):

- Equilibrate samples to room temperature (15-25°C).
- Equilibrate Buffer AVE to room temperature for elution step 10.
- Check that Buffer AW1, Buffer AW2, and carrier RNA have been prepared according to the instruction.
- Redissolve precipitate in Buffer AVL/ Carrier RNA by heating, if necessary , and cool to room temperature before use.
- All centrifugation steps are carried out at room temperature.

1- **Pipet 560 ul of prepared Buffer AVL containing Carrier RNA into a 1.5 micro centrifuge tube.**

If the sample volume is larger than 140 ul, increase the amount of Buffer AVL/ Carrier RNA proportionally (e.g., a 280-ul sample will require 1120 ul Buffer AVL/ Carrier RNA).

2- **Add 140 ul plama, serum, urine, cell-culture supernatant, or cell-free body fluid to the Buffer AVL/Carrier RNA in the microcentrifuge tube. Mix by pulse-vortexing for 15 sec.**

To ensure efficient lysis, it is essential that the sample is mixed thoroughly with Buffer AVL to yield a homogeneous solution. Frozen samples that have only been thawed once can also be used.

3- **Incubate at room temperature (15- 25 c) for 10 min.**

Viral particle lysis is complete after lysis for 10 min at room temperature. Longer incubation times have no effect yield on the yield or quality of the purified RNA. Potentially infectious agents and RNases are inactivated in Buffer AVL.

4- **Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.**

5- **Add 560 ul of ethanol (96- 100%) to the sample, and mix by pulse-vortexing for 15 sec. After mixing ,briefly centrifuge the 1.5 microcentrifuge tube to remove drops from inside the lid.**

Only ethanol should be used since other alcohols may result in reduced RNA yield and purity. If the sample volum is greater than 140 ul, increase the amount of ethanol proportionally (e.g., a 280-ul sample will require 1120 ul of ethanol). In order to ensure efficient binding, it is essential that is mixed thoroughly with the ethanol to yield a homogeneous solution.

6- **carefully apply 630 ul of the solution from step 5 to the QlAamp spin column (in a 2-ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min . place the QlAamp spin column into a clean 2-ml collection tube , and discard tube containing the filtrate.**

Close each spin colum in order to avoid cross-contamiation during centrifugtion. Centifugation is performed at 6000 x g (8000 rpm) in order to limit microcentrifuge noise. Centrifugation at full speed will not affect the yield or purity of the viral RNA. If the solution has not complete passed through.

7- **Carefully open the QIAamp spin column, and repeat step 6.**

If the sample volum was greater than 140 ul, repeat this step until all of the lysate has been loaded onto the spin column.

8- **Carefully open the QIAamp spin colum, and add 500 ul of Buffer AW1. Close the cap, and centrifuge at 6000 x g (8000 rpm)for 1 min. place the QLAamp spin column in a a clean 2-ml collection tube (provided), and discard the tube containing the filtrate.**

It is not necessaray to increase the volum of Buffer AW1 even if the original sample volume was larger than 140 ul.

9- **Carefully open the QIAamp spin column ,and add 500 ul of Buffer AW2. Close the cap and centrifuge at full speed (20,000 x g, 14,000 rpm) for 3 min. continue directly with step 10,or to eliminate any chance of possible Buffer AW2 carryover , perform step 9a, and then continue with step 10.**

Note: Residual Buffer AW2 in the eluate may cause problems in downstream a pplication. Some centrifuge rotors may vibrate upon deceleration, resulting in flow-through, contacting the QLAamp spin column,Removing the QLAamp spin colum and collection tube from the roror may also cause flow through to com into contact with the QLAamp spin column . in these cases , the optional step 9a should be peformed.

9a. **(Optional): place the QLAamp spin column in a new 2-ml collection tube (not provided),and discard the old collection tube with filtrate. Centrifuge at full speed for 1 min.**

10- **Place the QIAamp spin column in a clean 1.5- ml microcentrifuge tube (not provided). Discard the old collection tube containing the filtrate. Carefully open the QLAamp spin column and add 60 ul of Buffer AVE equilibrated to room temperatue. Close the cap, and incubate at room temperature for 1 min. centrifuge at 6000 x g (8000 rpm) for 1 min.**

A single elution with 60 ul of Buffer AVE is sufficient to elute at least 90% of the viral RNA from the QIAamp spin column. Performing a double elution using 2 x 40 ul of Buffer AVE will increase yield by up to 10% Elution with volumes of less than 30 ul will lead to reduced and will not increase the final concentrtion of RNA in the eluate. Viral RNA is stable for up to one year when stored at -20° C or -70°C . ***(QIAamp Viral HCV-RNA Mini Spin Protocol, 1999)***.

2) Amplification:

-One step RT – PCR Using QIAGEN one step RT- PCR kit (100)# 210212.

(Master Mix)

Component	1 X	••••X
RNase – Free Water	16 ul	
5X Qiagen One Step RT-PCR Buffer	10 ul	
d NTP Mix	2 ul	
Primer (6A)*	5 ul	
Primer (6B)*	5 ul	
Qiagen One Step RT-PCR Enzyme Mix	2 ul	
Templete Rna	10 ul	
Total volume	50 ul	

*** Primers most commonly used in hepatitis C.**

(Thermal Cycler Conditions)

Temp.	Time	Cycles
50 °C	30 min	1
95 °C	15 min	1
94 °C	1 min	40 cycles
55 °C	1 min	
72 °C	1 min	
72 °C	10 min	1
4 °C	Hold	

3) Detection

The expected PCR product for HCV is 270 base pair on 2% agarose gel with TAE buffer ***(QIAamp Viral HCV-RNA Mini Spin Protocol, 1999)***.

Statistical analysis

Data collected in this study were statistically processed using SPSS PC computer packing under windows on IBM compatible personal computer. Test for significance was done using propability value (P value); data were found to be normally distributed, so parametric tests (Mean value and SD) were applied. ***(Barbara,2002)***.

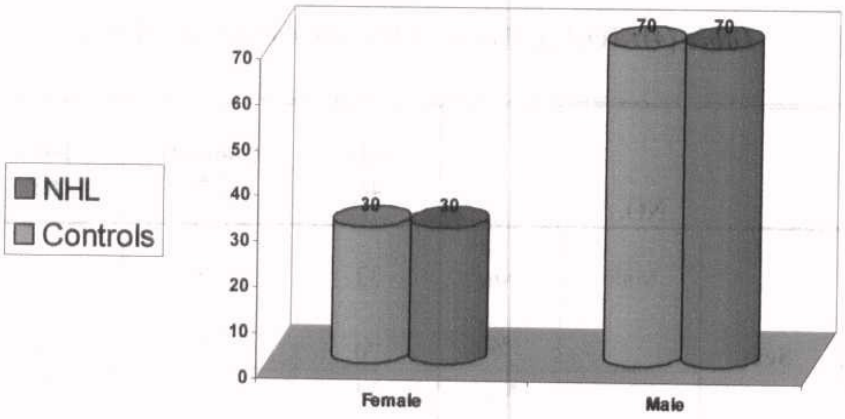
Results

Table (4): Total number, sex and locality of the studied cases.

NO.			NHL 46	Controls 37	Total 83
Sex	Male	**No.**	32	26	58
		%	70	70	70
	Female	**No.**	14	11	25
		%	30	30	30
	M:F	Ratio	2.3:1	2.3:1	2.3:1
Locality	Dakahlia	**No.**	36	30	66
		%	78.3	81	78.9
	Others	**No.**	10	7	17
		%	21.7	19	21.1

Table (4):- Shows total number, sex incidence and locality of cases of Non Hodgkin Lymphoma and Control cases. The mean age of the cases was (6.68) years, ranging from 2 years to 16 years and standard deviation 3.51. Male to female ratio of the studied cases in both groups was (2.3) to (1).

Fig 4 : Showing relation between NHL cases and control cases in sex, locality.



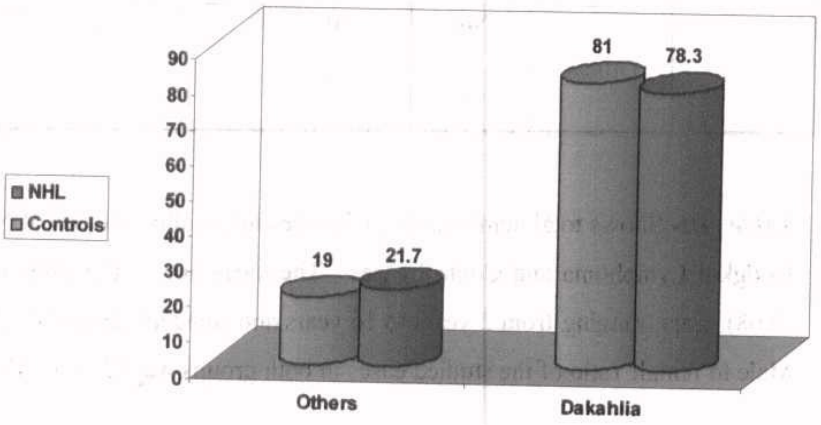


Table (5): Sex and Patholgy of NHL cases.

			Total NHL cases	New cases	Old cases
No.			46	22	24
%			100	47.8	52.2
Sex	**Male**	No.	32	14	18
		%	70	63.6	39.1
	Female	No.	14	8	6
		%	30	36.4	13.2
Pathology	**Burkitt**	No.	25	8	17
		%	54.3	36.4	70.8
	Lympho-blastic	No.	6	4	2
		%	13	18.2	8.3
	Large cell	No.	4	3	1
		%	8.7	13.6	4.2
	Small cell	No.	6	4	2
		%	13	18.2	8.3
	Others	No.	5	3	2
		%	10.9	13.6	8.3

Table (5): Demonstrats the sex and pathology of NHL cases according to date of diagnosis (before or after begining the study). ***Old cases***: 24 cases were diagnosed as NHL before beginning the study during or finished thier treatment. ***New cases:*** 22 cases was diagnosed after beginning not started treatment yet.

Table (6): **Clinical presentation of the NHL cases.**

Clinical presentation		Total NHL cases	New cases	Old cases
		46	22	24
Abdominal pain	No.	16	9	7
	%	34.8	41	29.2
Abd. Enlargment	No.	15	6	9
	%	32.6	27.5	37.5
Respiratory distress	No.	6	2	4
	%	13	9	16.6
Lymphadenopathy	No.	5	2	3
	%	10.9	9	12.5
CNS manifestation	No.	2	1	1
	%	4.3	4.5	4.1
Other	No.	2	2	0
	%	4.3	9	0

Table (6):- Shows clinical presentation of the NHL cases. About 70 % of cases presented with abdominal symptoms (pain and enlargment).

Other clinical presentations are facial swelling (submandibular mass), and pyrexia of unknown origin was (4.3%) of cases.

Fig.5 : Clinical presentation of the NHL cases.

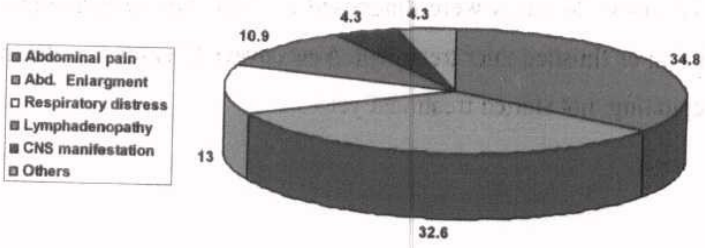


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Table (7): Clinical staging of the NHL cases.

Clinical staging		Total NHL cases	New cases	Old cases
		46	22	24
Stage 1	No.	0	0	0
	%	0	0	0
Stage 2	No.	5	2	3
	%	10.9	9.1	12.5
Stage 3	No.	37	19	18
	%	80.4	86.3	75
Stage 4	No.	4	1	3
	%	8.7	4.6	12.5

Table (7):- Shows the clinical staging of the NHL cases. 80.4 % of cases presented at stage III.

Fig 6: Clinical staging of the NHL cases.

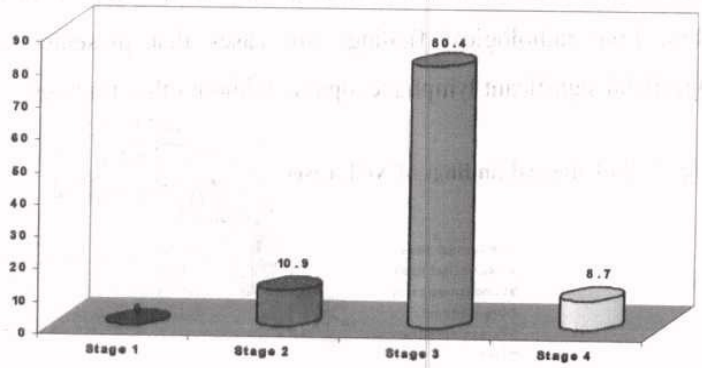

Table (8): Radiological findings of the NHL cases.

Radiological findings		Total cases	New cases	Old cases
		46	22	24
Abdominal mass	No.	31	12	19
	%	67.4	54.5	79.1
Mediastinal mass	No.	6	3	3
	%	13	13.6	12.5
Lymphadenopathy	No.	5	4	1
	%	10.9	18.2	4.2
Hepatosplenomegaly	No.	1	0	1
	%	2.2	0	4.2
Paraspinal mass	No.	1	1	0
	%	2.2	4.5	0
Free	No.	2	2	0
	%	4.3	9	0

Table (8):- Shows different radiological findings of the studied NHL cases. Routine radiological investigations were: 1- Chest x-ray 2- Abdominal ultrasonography. Then according to clinical presentation and findings in CXR or abd US , further radiological evaluation by C.T (abdomen or chest). MRI was needed only with 1 case with paraspinal mass. Free radiological findings are cases that presented only with superficial significant lymphadenopathy without other findings.

Fig 7: **Radiological findings of NHL cases.**

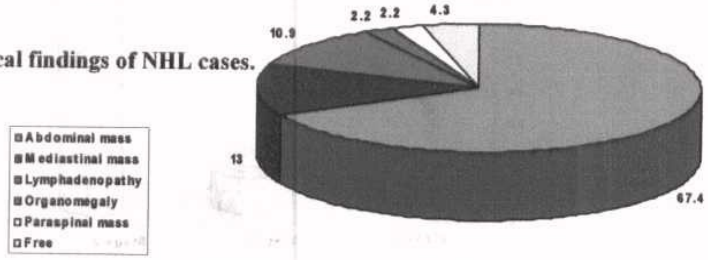

Table (9): Serum albumin and serum bilirubin of NHL group.

Laboratory finding	Serum Albumin		Serum Bilirubin	
	Normal > 2.9 g/dl	Hypoalbuminemia ≤ 2.9 g/dl	Normal ≤ 1.2 mg/dl	High > 1.2 mg/dl
NO. (46)	45	1	39	7
%	97.8	2.2	84.8	15.2

Serum albumin refrence value in children 2-16 years = 2.9 – 5.8 g/dl .
Serum bilirubin refrence value in children > 1 year = 0.3- 1.2 mg/dl
(THE HARRIET LANE HANDBOOK, 2005).

Table (9):- Shows levels of Serum albumin and serum bilirubin of the NHL cases . From the 7 cases with elevated serum bilirubin > 1.2 mg / dl ; we found only 1 case was a new case, the other 6 cases were old cases , thats may be due to the hepatotoxic cumulative effect of chemotherapy. The only case with hypoalbuminemia < 2.9 g / dl was a new case , she was presented to us with tense ascites and a huge intestinal mass .

Table (10): Liver enzymes of NHL cases.

Laboratory finding		ALT			AST		
		New cases 22	Old cases 24	Total 46	New cases 22	Old cases 24	Total 46
Normal	No.	19	13	32	19	18	37
	%	86.4	54.1	69.5	86.4	75	80.5
2 Folds	No.	2	5	7	1	2	3
	%	9.1	20.8	15.3	4.6	8.3	6.6
3 Folds	No.	1	4	5	1	1	2
	%	4.6	16.7	10.8	4.6	4.2	4.3
4Folds	No.	0	0	0	1	1	2
	%	0	0	0	4.6	4.2	4.3
> 4 Folds	No.	0	2	2	0	2	2
	%	0	8.4	4.4	0	8.3	4.3

ALT refrence value in children > 1 year = 7 – 40 U/L.

AST refrence value in children 2-18 years = 10 – 60 U/L

(THE HARRIET LANE HANDBOOK, 2005).

Table (10):- Shows levels of liver enzymes of NHL cases.

-Elevated ALT in 14 cases ≈ 30 %.

-Elevated AST in 9 cases ≈ 20 %.

Table (11): **Protocols of therapy of NHL group.**

Protocol of therapy	No. of cases	%
COMP protocol	43	93.5%
ESHAP Protocol	1	2.2%
Protocol of Acute Lymphoblastic Leukemia BFM 76/56	1	2.2%
APO Protocol	1	2.2%

Table (11):- Shows different protocols of chemotherapy of NHL cases. 43 cases (93.5%) recieved COMP protocol of treatment of NHL (Cyclofosphamide, Oncovin. Methotrexate and Prednisolone). 1 case recieved ESHAP protocol of agressive lymphoma (Etopside, Methyl prednisolone, Ara –C, Platinol). 1 case recieved the protocol of ALL due to bone marrow infilteration. 1 case recieved APO (Adriamycin, Prednisone, Oncovin, Methotrexate, 6-Mercapto purine) protocol for large cell lymphoma.

Table (12): Prevalence of HCV as detected by EIA 3.0.

Method			NHL			Controls
			New cases 22	Old cases 24	Total 46	37
EIA 3.0	+ve	*No.*	6	13	19	1
		%	27.3	54.2	41.3	2.7
	-ve	*No.*	16	11	25	36
		%	72.7	45.8	58.7	97.3
	P1 =		0.01*	0.001*	0.007*	
	P2=		0.74			

* ***P*** significant if<0.05 .

Chisquare test was used

P1 = Comparing each of the 3 groups with the control group.

P2 = Comparing new cases to old cases .

Table (12):- Shows HCV prevalence as detected by EIA 3.0 in the studied cases, Comparing the prevalence of HCV in new cases and old cases with control group. Higher prevalence of HCV in patients with NHL weather new ***(P=0.01*)*** or old cases ***(P=0.001*)*** and ***(P=0.007)*** in total NHL cases. Also compared prevalence of HCV between the new cases with old cases ***(P=0.74)*** which is insignificant relation.

Table (13): Prevalence of HCV as detected by RT-PCR .

Method			NHL			Controls
			New cases 22	Old cases 24	Total 46	37
PCR	+ve	*No.*	6	14	20	1
		%	27.3	58.3	43.3	2.7
	-ve	*No.*	16	10	26	29
		%	72.7	41.7	56.7	97.3
	P1 =		0.01*	0.001*	0.001*	
	P2=		0.69			

*P significant if<0.05.
Chisquare test was used

P1 = Comparing each of the 3 groups with the control group.

P2 = Comparing new cases to old cases .

Table (13):- Shows HCV prevalence as detected by nested RT-PCR in the studied cases, Comparing the prevalence of HCV in new cases and old cases with control group. Higher prevalence of HCV in patients with NHL wether new ***(P=0.01*)*** or old cases ***(P=0.001*)*** and ***(P=0.001)*** in total NHL cases. Also compared prevalence of HCV between the new cases with old cases ***(P=0.69)*** which is insignificant relation.

Fig (8): Prevalence of HCV as detected by EIA 3.0.

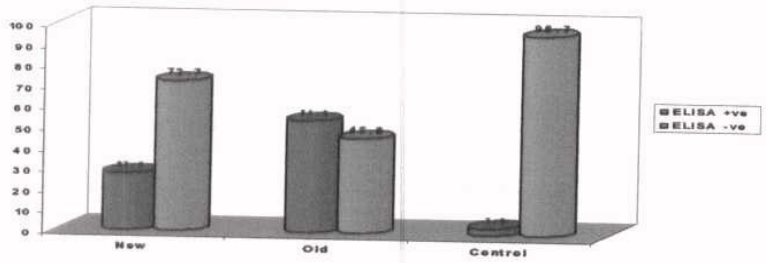

Fig (9) : Prevalence of HCV as detected by RT-PCR.

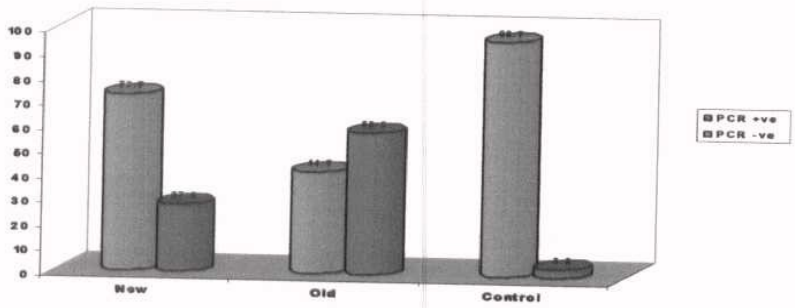

Fig. (10). Direct RT-PCR test photograph of the studied subjects.

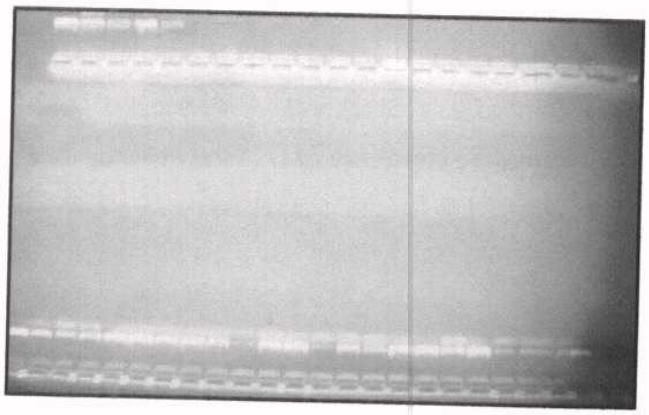

Table (14): Sex, liver functions and pathology of PCR +ve NHL cases.

		NHL				
PCR +ve cases *(20)*	***Sex***	Male		Female		
	No. %	17 85		3 15		
	Serum Albumin	Normal > 2.9 g/dl		Hypoalbumenimia ≤ 2.9 g/dl		
		NO %	20 100	0 0		
	Serum Bilirubin	Normal ≤ 1.2 mg/dl		High > 1.2 mg/dl		
		NO %	13 65	7 35		
	Liver enzymes			Normal	High	
		ALT	NO %	13 65	7 35	
		AST	NO %	15 75	5 25	
	Pathology	Burkitt	Lymphoblasic	Large cell	Small cell	Others
	No. %	13 65	5 25	2 10	0 0	0 0
	***P*=**	0.008*				

Table (14):- Shows the high significant relation between PCR +ve NHL cases and male sex. *(All PCR +ve new cases were males).*

-Shows the relation of PCR +ve NHL cases with liver function tests. *(only one PCR +ve new case with high serum bilirubin level >1.2 mg/dl).*

-Shows the high significant relation of PCR +ve NHL cases with Burkitts lymphoma with (***P***=0.008).

Table (15) : Clinical presentation and clinical staging of PCR +ve NHL cases.

	Clinical presentation			Clinical staging		
PCR +VE cases (20)	Abdominal pain	No. %	8 40	Stage 1	No. %	0 0
	Abd. Enlargment	No. %	6 30	Stage 2	No. %	1 5
	Respiratory distress	No. %	2 10			
	Lymphadenopathy	No. %	2 10	Stage 3	No. %	18 90
	CNS manifestation	No. %	1 5	Stage 4	No. %	1 5
	Others	No. %	1 5			

Table (15) : Shows the clinical presentation and clinical staging of PCR +ve NHL cases.

-The most common clinical presentations were abdominal symptoms; abdominal pain (40%) and abdominal enlargment (30%).

-Stage 3 was predominant in PCR +ve cases (90 %).

Table (16): **PCR relation with blood transfusion *before diagnosis*.**

			NHL	
			+ve Blood transfusion	**-ve Blood transfusion**
PCR +VE Cases (20)	***Total***	***No.***	15	5
	20	%	75	25
	New	***No.***	6	0
	6	%	100	0
	Old	***No.***	9	5
	14	%	64.3	35.7
PCR -VE Cases (26)	***Total***	***No.***	11	15
	26	%	42.3	57.7
	New	***No.***	7	9
	16	%	43.8	56.2
	Old	***No.***	4	6
	10	%	40	60

Table (16):- Showing that all HCV infected cases had past history of blood transfusion before being diagnosed as NHL. The 6 new NHL PCR +VE cases; 3 of them received blood transfusion during undergoing surgical operations; other 3 due to uncertain causes by their families. 9 of old cases had past history of blood transfusion before being diagnosed as NHL (as documented in their sheets).

Table (17): **PCR relation with blood transfusion *before the study.***

			NHL	
			+ve Blood transfusion	**-ve Blood transfusion**
PCR *+VE Cases (20)*	***Total*** 20	***No.***	20	0
		%	100	0
	New 6	***No.***	6	0
		%	100	0
	Old 14	***No.***	14	0
		%	100	0
PCR *-VE Cases (26)*	***Total*** 26	***No.***	12	14
		%	46.2	53.8
	New 16	***No.***	6	10
		%	37.5	62.5
	Old 10	***No.***	6	4
		%	60	40
Total 46	***No.***		32	14
	%		70	30

Table (17) : Showing that all the PCR +ve (20) cases had recieved blood transfusion before begining of study, also another 12 (6 new cases and 6 old) had recieved blood but had not catch HCV infection.

Discussion

Hepatitis C virus infection is a major health problem in Egypt, where the seroprevalence is more than 10% which is 10-20 fold higher than the united states and Europe ***(Frank et al., 2000; Soheir et al., 2002; El-Raziky et al., 2004)***. Infection with HCV results in viral persistence which posses a higher risk of infection to individuals who receive blood or blood products from HCV infected donors ***(Esteban et al., 1990)***.

Several infectious agents have been identified as possible etiologic agents for NHL; in most cases, the presence of a particular agent increases the risk of developing cancer or speeds its progression. For example, HIV and other viruses that affect the immune system make infected individuals prone to a variety of cancers by weakening the body's natural defenses. But in other cases, there is now compelling evidence that certain agents may also play a critical role in causing cancer. However, proving that a particular agent causes cancer is complicated. HCV is postulated to be involved in the pathogenesis of B-cell NHL. Yet have not been linked to a known translocation, nor endowed with oncogenes. The exact mechanism of neoplastic transformation is still unknown (Zucca et al., 2004).

The best-known infectious causes of NHLs are associated with specific subtypes of lymphoma (e.g., the retrovirus HTLV1 with acute T-cell leukemia/lymphoma, the Epstein-Barr virus (EBV) with endemic Burkitt's lymphoma and immunoblastic lymphoma in immunosuppressed patients, Helicobacter pylori with MALT lymphoma of the stomach) ***(Pagano, 2002)***. Some subtypes, notably lymphoplasmacytoid lymphoma/ immunocytoma showed especially elevated HCV prevalence ***(Mele et al., 2003)***.

Several studies have reported a high rate of prior hepatitis C viral infection in patients with non-Hodgkin's lymphoma (NHL). However, it appears that there are marked geographical differences in the prevalence of HCV among NHL patients. Further there is controversy concerning a possible pathogenetic link between HCV and certain histologic lymphoma subtypes, in particular MALT lymphomas and it has recently been speculated that HCV might be involved in the multistep process of gastric lymphoma genesis ***(De Vita et al., 2003)***.

We conducted this study in the period from November 2004 to November 2005. The study was applied on 83 subjects; 46 cases of NHL attending an oncology outpatient clinic at the MUCH, wether newly diagnosed cases or cases taking their chemotherapy or finished their treatment on follow up visits and 37 Control subjects free from cancer, were selected by sex and birth place from among all patients of the general outpatient clinic of the same hospital with negative past history of blood transfusion or surgical operations. The mean age of the subjects was (6.68) years, ranging from 2 years to 16 years and standard deviation 3.51. Male to female ratio of the studied cases in both groups was (2.3) to (1).

We collected samples from all the 83 subjects, then within 2 hours of collection, the blood was separated and the serum divided into aliquots and stored at 80° C. Samples were later thawed and tested for anti-HCV antibody by HCV enzyme-linked immunoassay (EIA) third generation (Equipar srl Via G.Ferrari, 21/N – 21047 saronno (Va) Italy), according to the manufacturer's instructions. Samples were then tested for HCV RNA by direct nested reverse transcription-polymerase chain reaction (RT-PCR) (QIAGEN S.p.A Via Grosio, 10/10 .20151 Milano Italy). A positive result by the direct RT-PCR method was considered truly positive, and no further

investigation was done. A sample that was negative by both direct RT-PCR and EIA was considered negative. High prevalence of HCV 20/46 (43.4%) was detected in NHL cases when compared to the 37 controls 1/37 (2.7%). Twenty patients were positive for HCV-RNA, 19 anti-HCV reactive patients and one patient was negative by anti-HCV (suspected due to high levels of liver enzymes at presentation). The only patient of the controls, who was anti-HCV positive, was found positive by PCR.

We encounterd some characteristics in NHL cases: 70% males, 78% from inside Dakahlia governrate, 70% with abdominal pain and enlargment, 13% with respiratory distress, 11% with lymphadenopathy, 55% of pathological subtype Burkitt's lymphoma, 80% in stage 3 of NHL, 69.5% were blood transfused before the study, 97.8% had normal serum Albumin, 84.4% had normal serum Bilirubin, 80.5% had normal AST level.

We encouterd some characteristics in NHL RT-PCR +ve cases: 85% males, 75% from inside Dakahlia governrate, 70% with abdominal symptoms (pain and enlargment), 65% of pathological subtype Burkitt's lymphoma ***(P=0.008)***, 90% in stage 3 of NHL, 100% blood transfused before the study, 100% with normal serum Albumin, 65% with normal serum Bilirubin, 75% with normal AST level.

Whether this increased prevalence of HCV indicates an active role in lymphomagenesis or it just represents a failure of eradication of infection as a result of weak immune system is difficult to decide on the basis of our results.

Primarily, the young age of patients does not allow enough time for the evolution of an established malignant disorder on top of an infectious disease, especially for an HCV infection. Although it was previously stated that HCV infection in NHL patients was associated with a shorter time to lymphoma progression ***(Turner et al., 2003)***, still this observation has yet to be verified in the pediatric group of patients.. Actually, the possibility exists that HCV infection occurred as independent events on a previously disturbed immune system that is susceptible to chronic infection and lymphomagenesis. Or, it is also possible that HCV infection were a previous event that led to immune suppression, making the patient more amenable for lymphoma to occur. In fact, the presence of HCV infection in a good percentage of patients necessitates a careful search for the effects of infection in this population of patients ***(Turner et al., 2003).***

In adult Egyptian NHL patients, almost one third of patients showed evidence of HCV infection, 32% for HCV-antibodies and 28% for HCV-RNA ***(Aboul-Enein et al., 2003).*** These results corresponded with another study from Italy in which HCV prevalence was 17.5% among 400 lymphoma patients and 5.6% among 396 controls ***(Mele et al., 2003).*** Furthermore, HCV infection was detected in 17/100 of patients with B-cell NHL versus 0/25 patients with non-B-cell NHL (p=0.023) and in 34 patients (6.6%) in the control group with miscellaneous diseases (p=0.0011) ***(Mizorogi et al.,2000)***. On the other hand, other studies conducted in areas where HCV is rare as France, failed to find an association between HCV and NHL (Hausfater et al., 2001).

It is noteworthy that many of the studies concerning HCV infection in NHL were done in adult patients. In some of these studies, HCV positivity was significantly associated with older age group ***(Catassi et al., 1998).*** The increased prevalence of HCV in pediatric NHL patients might

reflect the increased incidence of HCV in certain areas of the Egyptian population, as an incidence of 12% was previously reported in children living in rural areas ***(Abdel-Wahab et al., 1994)***. This is supported by the finding of genotype 4 in most of HCV-RNA positive patient, which is the predominant type found in Egypt population ***(Zekri et al., 2000)***.

There has been a proliferation of papers on the association between HCV and NHL. A general association of HCV and B-cell NHL has been reported in studies from Brazil ***(Chindamo et al., 2002)***, Italy ***(Mazzaro et al., 1996;De Rosa et al., 1997;Vallisa et al., 1999; Montella et al., 2001)***, Israel ***(Shirin et al., 2002)***, Japan ***(Mizorogi et al., 2000; Kuniyoshi et al., 2001)***, Romania ***(Cucuianu et al., 1999)***, Turkey ***(Paydas et al., 1999)***, Switzerland ***(Zucca et al., 2004)*** and the USA ***(Zuckerman et al., 1997)***. Some of these studies have used inappropriate control groups, such as healthy blood donors, which potentially confound interpretation, and those studies are not discussed here. Studies that have used more appropriate control groups, including those from Italy ***(De Rosa et al., 1997;Vallisa et al., 1999; Montella et al., 2001)***, Japan ***(Mizorogi et al., 2000)*** and the USA ***(Zuckerman et al., 1997)***, have found a general association of B-cell NHL and chronic infection with HCV. A further paper from Italy has produced equivocal results ***(Pioltelli et al., 2000)***.

The epidemiological data supporting a general association of HCV and lymphoma remains controversial, with considerable discordance between reports. The majority of positive studies have originated in Italy, where the prevalence of HCV is particularly high, with reported prevalences of up to 2.9% in parts of the north of the country ***(Bellentani et al., 1994)***, and up to12.6% in parts of the south ***(Guadagnino et al., 1997)***. The lymphoma with the clearest link to HCV is lymphoplasmacytoid lymphoma, an overt B-cell lymphoma that can complicate Essintial Mixed

Cryoglobinemia (EMC), with up to 30% of cases associated with hepatitis C ***(Ferri et al., 1994;Silvestri et al., 1998)***. These initial studies compared the rate of HCV antibodies in retrospective cohorts of lymphoma patients with the healthy background population as control. It was not until larger case–control studies had been performed that a more general association with other B-cell malignancies was found.

The finding of an association is not universal, with a large case–control study from France ***(Hausfater et al., 2001)***, and multiple small negative series from the UK ***(McColl et al., 1997)***, Canada ***(Collier et al., 1999;Shariff et al., 1999)***, Germany ***(Ellenrieder et al., 1998)***, Turkey ***(Kaya et al., 2002)***, Thailand ***(Udomsakdi et al., 2000)*** and the USA ***(Rabkin et al., 2002)*** failing to find an association. In contrast to the Japanese studies supporting the association, a further Japanese study found little evidence of a significant association ***(Ohsawa et al., 1999)***.

Why might these studies have failed to find an association?,Clearly one possible reason would be that there is no association between lymphoma and HCV. Another reason may be inaccurate interpretation, Many positive studies are well conducted, however, and other reasons are likely to explain the discrepancies between studies. At best, only a small minority of HCV carriers will develop lymphoma, otherwise countries with a high prevalence of HCV carriage would be overwhelmed by cases of lymphoma. An accurate assessment of the exact risk could only come from a large cohort study, and no such studies have been reported. Many of the negative studies have been small, , or have studied populations with a low prevalence of HCV carriage, and hence have only been able to identify a weak association ***(Hausfater et al., 2001)***.

It is clear that the apparent discrepancy between the various studies performed so far could be explained by many possible reasons including:

i) Different genetic, ethnic and environmental characteristics in the populations studied.

ii) Variability of genotypes and subtypes of HCV, possibly related with the geographic distribution and the natural history of the infection.

iii) Different prevalences of cryoglobulinemia in Europe, North America and South America.

iv) Different results according to the methods used to evaluate the prevalence of HCV infection.

v) Lack of uniformity in the classification of lymphomas and

vi) Different distributions of NHL histologic subtypes ***(Ferri et al., 1995;Lonardo et al., 1995)***.

The evidence of HCV infection in bone marrow and in hematopoietic CD34+ pluripotent cells indicates that HCV replication occurs in the initial stages of the differentiation of progenitor hematopoietic cells. Recently a specific receptor (CD81) was described in hepatocytes and B lymphocytes as the putative bonding site of the E2 protein of HCV envelope, and this could represent the missing link between the known effects of HCV in the liver and its possible effects on lymphocyte proliferation ***(Pileri et al., 1998).***

Both Hodgkin's lymphoma and T-cell NHL consistently show no association with HCV. There may be an association of myeloma with HCV, in addition to B-cell lymphomas. Although initial studies on HCV associations focused on lymphocytoplasmacytoid lymphoma as the principal HCV-associated lymphoma ***(Ferri et al., 1994)***, this lymphoma is not prominent in many later series. In some series, low grade histologies predominate, but in other series the association is with intermediate- or high-grade lymphomas ***(Mizorogi et al., 2000;Shirin et al., 2002).***

The presentation of NHL associated with HCV differs from standard NHL. Lymphomas associated with HCV more commonly present as primary extra-nodal lymphomas ***(De Vita et al.,1997;Satoh et al., 1997; Montella et al., 2001)***. Also, 65% of diffuse large B-cell lymphomas associated with HCV may present as primary extra-nodal lymphomas, compared with 19% of controls ***(De Vita et al., 1997)***. This mirrors the ability of the HCV to infect these organs ***(De Vita et al., 1995)***. Cryoglobulinaemia is commonly found in HCV-associated lymphomas, especially lymphoplasmacytoid lymphoma ***(Silvestri et al., 1998)***.

Thus, we report a high prevalence of HCV infection in pediatric NHL patients at MUCH 20/46 (43.4%). Epidemiological evidence strongly suggests a link between chronic infection with hepatitis C and B-cell NHL. Etiological relationship could not be postulated on the ground of the results of the present study, no direct evidence to support or refuse this relationship. Transfusion of blood and/or blood products are the major risk factors in the devolepment of hepatitis C. From the available evidence it appears that HCV is an important risk factor for NHL in areas with a high prevalence of HCV .

Summary and conclusion

Summary

The relationship between HCV infection and lymphoproliferative diseases was first reported by ***Ferri et al in 1994*** and was subsequently highlighted by other authors, with the prevalence of HCV infection ranging from 9 - 42%.

We conducted this study to determine the prevalence of HCV infection in children with NHL in Mansoura University children Hospital. Eighty three subjects were enrolled in our study in two groups; Cases group of 46 NHL cases and control group of 37 cases. Then , sample of each subject tested for anti-HCV antibody by HCV third generation enzyme-linked immunoassay (EIA 3.0) and Samples were then tested for HCV RNA by direct nested reverse transcription-polymerase chain reaction (RT-PCR) aiming to determine HCV prevalence among these subjects, a positive result by the direct RT-PCR method was considered truly positive, and no further investigation was done. A sample that was negative by both direct RT-PCR and EIA 3.0 was considered negative.

A high prevalence of HCV 20/46 (43.3%) was detected in pediatric NHL patients when compared to the control group 1/37 (2.7 %) in healthy children

Conclusion

Epidemiological evidence strongly suggests a link between chronic infection with hepatitis C and B-cell NHL in high prevalent areas.

We conclude that:-

1- Our finding of a significant association between HCV infection and NHL which postulates the lymphotropic affinity of HCV, has lead us to suggest that HCV is an important risk factor for NHL in areas with high prevalence of HCV, thus; investigation should be performed routinely in such cases at diagnosis .

2- Transfusion of blood and/or blood products are the major risk factors in the devolepment of hepatitis C.

3- Male sex has higher risk to catch HCV infection and to devolop NHL.

4- Burkitt pathological subtype (Small non cleaved B-cell) of NHL is endemic in our region and it has significant relation with HCV infected patients.

In conclusion, the findings of our study make us consider that there is an association between HCV infection and the occurrence of neoplastic NHL in our region. Further studies are required to evaluate the precise role of HCV in the multistep process leading to monoclonal proliferation ending with NHL.

Recommendation

From the results of this review we recommended that:

1- HCV investigation should be performed routinely in all newly diagnosed NHL cases.

2- Further studies including the detection of the immune system components, in addition to investigating other infections are needed to clarify whether there is a defective immune system responsible for both chronic infections and lymphoma or that there is an actual relationship between these agents and pediatric lymphomas.

3- Accurate screening of blood products for HCV, Use of disposable equibments, and Health education of paramedical personnel.

4- Management of hepatitis C during treatment of Lymphoma with antivirals may help in the success of treatment. Avoiding periods of stopping chemotherapy due to combined viral and toxic hepatitis (which may increase the propability of recurence of lymphoma).

5- Cooperation and exchange of information between different oncology centers and via special recording system.

6- Proper documentation, data collection and analysis of patients from different localities and all over the country.

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Arabic Summary

الملخص العربي

المقدمة:

وجدت منظمة الصحة العالمية أن أكثر من ١٧٠مليون شخص على مستوى العالم، و ٨-١٠ مليون من المصريين (١٢-١٥٪) يعانون من التهاب الكبد الفيروسى سى (أتش سى فى).

أول من درس العلاقة بين الأورام الليمفاوية الخبيثة مع عدوى فيروس سى كان الإيطالي فيررى فى عام ١٩٩٤ ، ثم علماء آخرين و تراوحت نسبة انتشار فيروس سى فى حالات الأورام الليمفاوية فى هذه الدراسات بين ٩-٤٢٪ .بينما فى دراسات أخرى عن نفس العلاقة فى المملكة المتحدة و الولايات المتحدة الأمريكية لم تجد أى علاقة، و هذا يدل على تأثير نسبة انتشار فيروس سى فى أى منطقة على هذه العلاقة.

تم عمل دراسة إحصائية عن هذه العلاقة فى مستشفى الأطفال الجامعى – المنصورة – محافظة الدقهلية على جميع حالات الأورام الليمفاوية غير هودجكن، لمعرفة مدى انتشار فيروس سى فى هذه المنطقة.

الهدف من البحث:

١- دراسة نسبة أنتشارعدوى فيروس سى و الأورام الليمفاوية الخبيثة.
٢- أعطاء دراسة أحصائية فى مصر عن أنتشار فيروس (سى) فى مرضى الأورام الليمفاوية عند الأطفال.

دراسة هذه العلاقة فى منطقة عالية الأنتشار بفيروس سى مثل مصر يفيد بأن نقدم دراسة أحصائية وافية.

طريقة البحث:

وقد أجرى هذا البحث على ٤٦ مريضا مصابون بالأورام الليمفاوية تتراوح أعمارهم بين ٢-١٦ عام، ٣٢ ذكر و ١٤ أنثى بنسة ٢.٣:١ بمستشفى الأطفال الجامعى- المنصورة:

- ٢٢ حالة بالليمفوما غير هودجكين تم تشخيصهم بعد بداية الدراسة.
(حالات جديدة، لم تبدأ العلاج).
- ٢٤ حالة بالليمفوما غير هودجكين تم تشخيصهم قبل بداية الدراسة.
(حالات قديمة، أثناء فترة العلاج أو المتابعة).

و مقارنتهم مع ٣٧ من الأطفال الأصحاء من العيادة العامة الخارجية لنفس المستشفى، و اشترط فيهم عدم إجراء نقل دم أو عمل أى عملية جراحية من قبل.

وقد تم لجميع هؤلاء المرض عمل :

- ملف كامل به كل المعلومات المفيدة للبحث،
- وظائف كبد كاملة.
- اختبار الأجسام المضادة.
- تفاعل اليلمرة المتسلسل.

نتائج البحث:

-وجدنا انتشار فيروس ج بصورة كبيرة (٤٣٪) بين أطفال الأورام الليمفاوية، بالمقارنة مع (٢.٧٪) للأطفال الأصحاء.

-هذه النتيجة ترجح وجود علاقة قوية بين فيروس ج و الأورام الليمفاوية غير هودجكين، واحتمال عمل فيروس ج على الخلايا الليمفاوية و تحويلها لخلايا سرطانية.

التوصيات:

-إجراء التحاليل الخاصة بالكشف عن فيروس ج عند أى حالة أورام ليمفاوية جديدة.

-إجراء دراسات أخرى عن مدى تأثير فيروس ج على الجهاز المناعى للجسم.

-الإرشاد الصحى السليم عن وسائل انتشار عدوى فيروس ج.

-التعاون المشترك و تبادل المعلومات بين جميع مراكز الأورام عن طريق نظام موحد لتسجيل بيانات الحالات.

كلية الطب
قسم طب الأطفال

دراسة عن علاقة فيروس سي و نسبة انتشاره في الأورام الليمفاوية الخبيثة عند الأطفال

مجزء من المتطلبات للحصول على درجة الماجستير في طب الأطفال

رسالة مقدمة من

محمد محمود السيد الناغي
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